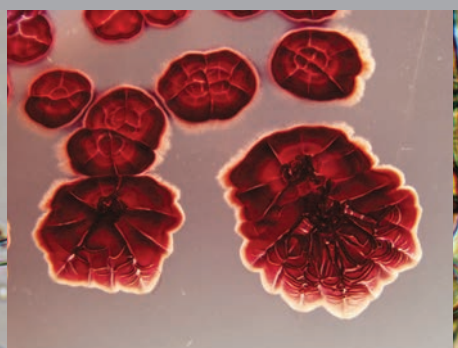
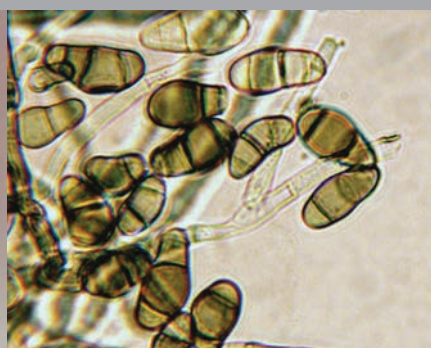
Ecology of
Soil Microorganisms
2015

Abstract Book

Ecology of Soil Microorganisms

Microbes as Important Drivers of Soil Processes

29. 11.–3. 12. 2015, Prague, Czech Republic

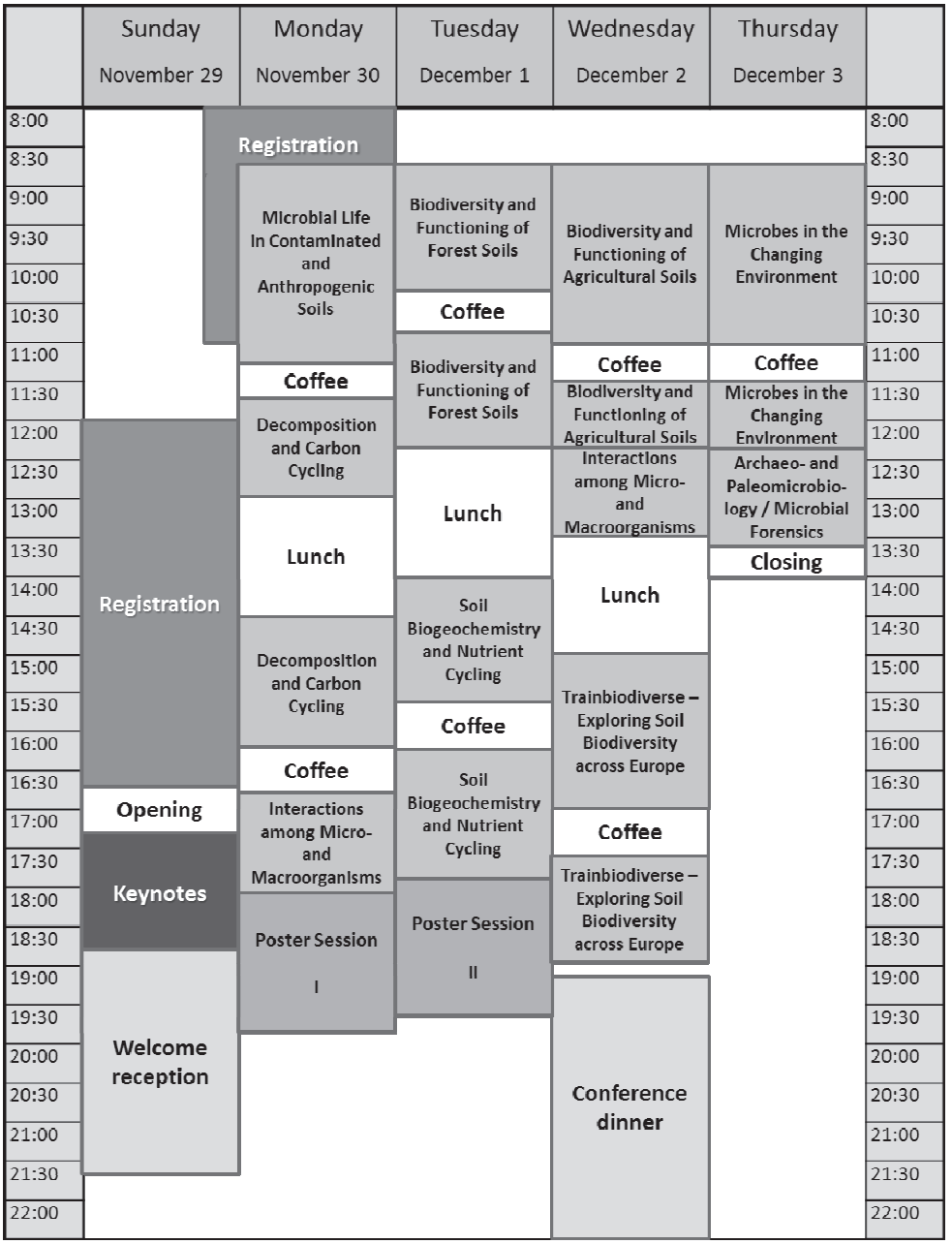




Ecology of Soil Microorganisms 2015

November 29 – December 3, 2015

Prague, Czech Republic

BOOK OF ABSTRACTS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Registration
Microbial Life In Contaminated and Anthropogenic Soils
Coffee
Decomposition and Carbon Cycling
Lunch
Decomposition and Carbon Cycling
Coffee
Interactions among Micro- and Macroorganisms
Poster Session I
Biodiversity and Functioning of Forest Soils
Coffee
Biodiversity and Functioning of Forest Soils
Lunch
Soil Biogeochemistry and Nutrient Cycling
Coffee
Soil Biogeochemistry and Nutrient Cycling
Poster Session II
Biodiversity and Functioning of Agricultural Soils
Coffee
Biodiversity and Functioning of Agricultural Soils
Interactions among Micro- and Macroorganisms
Lunch
Trainbiodiverse – Exploring Soil Biodiversity across Europe
Coffee
Trainbiodiverse – Exploring Soil Biodiversity across Europe
Conference dinner
Microbes in the Changing Environment
Coffee
Microbes in the Changing Environment
Archaeo- and Paleomicrobiology / Microbial Forensics
Closing
Registration
Opening
Keynotes
Welcome reception

WELCOME

We are pleased to welcome you to the second conference on the Ecology of Soil Microorganisms to be held in November/December 2015 in Prague. This is the second conference on the same topic after the first one organised in 2011 and we are happy to see that it has again attracted the attendance of more than 400 participants from all over the world. The conference should represent an interdisciplinary platform that offers as much interaction among various subjects within microbial ecology as possible. This includes questions addressing individual microbes, microbial communities as well as their interactions with the environment and other soil biota. We hope to link the modern molecular "omics" methods such as metagenomics, metatranscriptomics and metaproteomics with approaches based on soil chemical and biochemical analyses, the exploration of soil fauna and plant ecology. The other important goal of the conference is a wide scope covering the ecology of all microbes: bacteria and fungi as well as archaea and protozoa. Our aim is to bring experts from all these disciplines to a meeting where all can benefit from interactions and to promote in this way the research in the field of soil ecology.

We hope that you enjoy the programme of invited and contributed oral talks as much as we have enjoyed putting it together for you. Unfortunately, the limited time for oral contributions has resulted in several extremely interesting topics having to be presented as posters. Please do not miss either of the two poster sessions that considerably increase the diversity of the topics covered.

We would like to acknowledge the support of all those who have contributed to the conference organization, our sponsors, exhibitors and the members of our scientific committee. However, the major thanks go to all of you – the contributors to our rich five-day scientific programme. The conference is the fruit of your efforts, so please, enjoy it!

Petr Baldrian
Chair of the Organizing Committee

GENERAL INFORMATION

Organising commitee

Petr Baldrian - chair
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Members
Michael Schloter (Helmholtz Centre, Munich), Vendula Brabcová (Institute of Microbiology of the ASCR, Prague), Tomáš Cajthaml (Charles University, Prague), Alica Chroňáková (Biology Centre ASCR, České Budějovice), Anna Davidová (Institute of Microbiology of the ASCR, Prague), Dana Elhottová (Biology Centre ASCR, České Budějovice), Jan Frouz (Biology Centre ASCR, České Budějovice), Markéta Marečková (Crop Research Institute, Prague), Martin Pospíšek (Charles University, Prague), Hana Šantrůčková (University of South Bohemia, České Budějovice),Martina Štursová (Institute of Microbiology of the ASCR, Prague), Tomáš Větrovský (Institute of Microbiology of the ASCR, Prague)

Scientific committee
Petr Baldrian (Czech Republic)
Lynne Boddy (United Kingdom)
Jan Frouz (Czech Republic)
George Kowalchuk (The Netherlands)
Paolo Nannipieri (Italy)
Michael Schloter (Germany)
Christoph Tebbe (Germany)
Jan Dirk van Elsas (The Netherlands)

Keynote lectures
Michael Wagner (Austria): Function first: New ways to study soil microbes on a single cell level
Francis Martin (France): Harnessing genomics for understanding tree-microbe interactions in forest ecosystems

Invited speakers
Jeremy Austin (Australia), Erland Bååth (Sweden), Petr Baldrian (Czech Republic), Lynne Boddy (United Kingdom), Michael Bonkowski (Germany), Bruce Budowle (United States of America), Tomáš Cajthaml (Czech Republic), Jeremy Austin (Australia), Jan Frouz (Czech Republic), Ellen Kandeler (Germany), George Kowalchuk (The Netherlands), Björn Lindahl (Sweden), Mari Moora (Estonia), David Myrold (United States of America), Paolo Nannipieri (Italy), James I. Prosser (United Kingdom), Marc-André Selosse (France), Angela Sessitsch (Austria), Christa Schleper (Austria), Michael Schloter (Germany), Kornelia Smalla (Germany), Søren J. Sørensen (Denmark), Christoph Tebbe (Germany), Leho Tedersoo (Estonia), Jan Dirk van Elsas (The Netherlands), Timothy Vogel (France)

GENERAL INFORMATION

Conference organisation
Institute of Microbiology of the Czech Academy of Sciences
Biologicals
Conference website
http://www.soilmicrobes.org

Conference Venue
TopHotel Prague, Blažimská 1781/4, Praha 4, Czech Republic
http://www. tophotel.cz/en

To reach the venue by public transport, take the metro to the station „Chodov" and from there take the bus No 115 to the station "Městský archiv" (10 min). Bus departures from „Chodov" are available at:
http://jrportal.dpp.cz/jrportal/Down.aspx?f=/DataFTP\JRPortalData/115/20141214/115_(52_4)T.pdf, departures from TopHotel at:
http://jrportal.dpp.cz/jrportal/Down.aspx?f=/DataFTP\JRPortalData/115/20141214/115_(1132_1)Z.pdf.
Please refer to the map for detail. Paid public parking is available on site.

Language
The official language of the conference is English.

Registration
Registration desk in Top Hotel Praha will be open in a foyer of the Top Congress Hall. Opening hours:

Sunday, Novemberl 29	12:00-18:00
Monday, November 30	8:00-11:00
Tuesday,December 1 – Thursday, December 3	8:00-9:00

All participants will receive Certificate of attendance during registration.

Name badges
All conference participants, accompanying persons and exhibitors are kindly requested to wear their badges throughout the conference in order to be admitted to the lecture hall and other conference facilities. Name badges will be also required for the admission to the Gala Dinner.

Oral presentations
All oral presentations will be held in the Top Congress Hall.
Invited talks are scheduled for 22 minutes + 3 minutes for questions, contributed talks for 12 minutes + 3 minutes for questions. The talks in the "Trainbiodiverse" session will be for 10 minutes + 2 minutes for questions. Presentations should be prepared in Microsoft PowerPoint 2010-compatible format. The authors are responsible for checking the functionality of their presentations. The presenters' computer will have an Internet access but we strongly recommend saving any web pages in the presentation offline. Speakers' ready corner will be

available to all speakers from the beginning of registration until the end of the day's program. The presentations should be submitted at latest one day before they are scheduled to the organizers in the Speakers' ready corner.

Poster presentations
Poster presentations will be displayed in and around the Top Congress Hall. POSTER NUMBERS CORRESPOND TO THE PAGE NUMBERS IN THIS ABSTRACT BOOK. All posters will be displayed throughout the whole conference, from Sunday, November 29 until Thursday, December 3. The posters can be pinned up from 15:00 on Sunday and must be collected before 14:00 on Thursday. Pins will be provided by the organizers. Poster dimensions should not exceed 90 cm (width) x 150 cm (height). Posters with odd numbers will be presented by their authors during Poster session I (Monday), posters with even numbers during Poster session II (Tuesday). Presenting authors are requested to be available at their posters for informal discussion during their poster session.

The organizers are not responsible for any posters that have not been removed in time.

Student poster awards (100 EUR + diploma) will be awarded to five best posters presented by authors that are students. The winners will be announced on December 2 before Lunch.

Internet access
Wireless internet will be available in the conference hotel free of charge to all participants.

Exhibitions
Commercial exhibition will take place during the conference in the lobbies around the Top Congress Hall.

Refreshments
Lunches (hot buffet, Monday - Wednesday) and coffee breaks (coffee and tea) are included in the registration fee. Lunches will be served in the Hall Praha, coffee will be served in the foyer. Name badges should be used during lunches and coffee breaks. Please note that „Ecology of Soil Microorganisms" is a nonsmoking conference.

Wellcome reception
All conference participants are cordially welcome to join the Welcome reception to be held on Sunday, November 29 from 19:00 till 21:30 in the Hall Praha. The reception is included in the registration fee.

Gala dinner
Gala dinner will take place in the Hall Praha on Wednesday, November 2 between 19:15-22:15. Tickets ordered by the participants will be available at the registration desk. A limited number of tickets will be available for purchase at the registration desk for 1650 CZK.

Liability and Insurance

The organizer is not able to take responsibility whatsover for injury, damage or loss involving persons and property during Conference. Participants are advised to take out their own personal travel and health insurance for their trip.

PRACTICAL INFORMATION

Public transport in Prague
There are three metro lines and a number of tramway and bus lines in Prague. Details on city transport system are available at the official Prague Public Transport web site http://www.dpp.cz/en/.

Tickets
Tickets are sold at yellow ticket machines (also in English, coins only), at ticket offices located at some metro stations, in newspapers stands, and at tourist information centers. Each ticket must be validated upon the entrance to the metro station or in the tram / bus to mark the start of the validity period.

Tickets cost 24 CZK (30 min) or 32 CZK (90 min since validation). It is possible to change between buses, trams and metro. This ticket is not valid on night trams and buses, on the Petřín funicular and on ferries. Ticket can be paid by SMS.

Taxi
You can find the contacts for recommended non-stop taxi call centres in Prague at the official website of Prague: http://www.praguewelcome.cz/en/

Currency
The currency is the Koruna (CZK). 1 EUR is approximately 28 CZK, 1 USD is approximately 25 CZK. Money can be exchanged at numerous exchange offices and in banks, as well as in some hotels, shops and restaurants, Euros can be often used as well (ask in advance). In most shops and restaurants, credit cards can be used.

Emergency call
112

Call to the Czech Republic
The international country code of the Czech Republic is 420 (dial either 00420 or +420 if not already included at the beginning of the telephone number).

Electricity
230 Volts/50 Hz, type E electrical outlet. You can buy plug converters in electronic stores.

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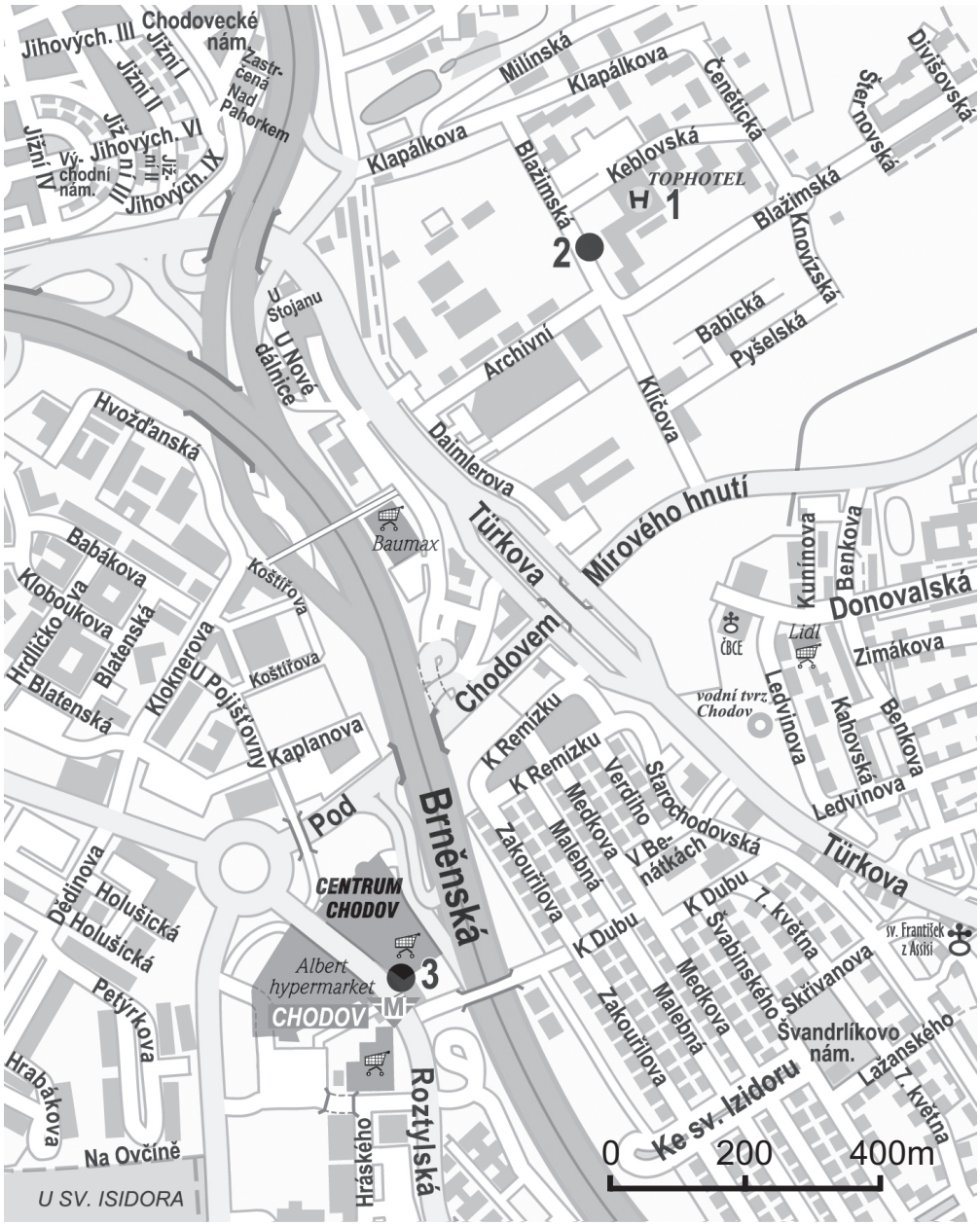
CONFERENCE VENUE AND SURROUNDINGS



1 – TopHotel Prague, conference venue
2 – Bus stop of bus 115 “Městský archiv”
3 – Bus stop of bus 115 “Chodov” going to the conference venue; transfer from / to metro line C, shopping centre

Sponsors, Exhibitors and Partners

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Pyro Science GmbH

Roche

Terragenome

Trainbiodiverse

SUNDAY, 29 NOVEMBER 2015

12:00-16:30 Registration

Opening session (Michael Schloter)

16.45 **Welcome** - Petr Baldrian

17:15 **Keynote:** Michael Wagner: Function First: New Ways to Study Soil Microbes on a Single Cell Level

18:00 **Keynote:** Francis Martin: Unearthing the Roots of Fungal Symbioses

19:00-21:30 Welcome reception

MONDAY, 30 NOVEMBER 2015

8:00-11:00 Registration

Microbial Life in Contaminated and Anthropogenic Soils (Christoph Tebbe)

8:45 Michael Schloter: Reconstruction of microbial nutrient cycles in soil using metagenomic approaches (Invited lecture)

9:10 Timothy Vogel: Microbial Social Networks in Contaminated Soils (Invited lecture)

9:35 Tomáš Cajthaml: Insights into ecology during microbial bioremediation as assessed through advanced techniques – case studies (Invited lecture)

10:00 Matthias Kästner: Degradation of ^{13}C-labelled pyrene in soil-compost mixtures and farmyard fertilized soil – turnover mass balances and community analyses

10:15 Jennifer Wiltshire: Microbial community dynamics in the rhizosphere of a heavy-metal hyper accumlator

10:30 Anja Worrich: Impact of mycelia-like dispersal networks on bacterial spatiotemporal dynamics linked to biodegradation at varying water potentials

10:45 Antonios Michas: Soil - sediment microbial community adaptation due to a long history of oil contamination

11:00 Anne Winding: Biochar for soil carbon sequestration and P fertilization: effects on microbes and fauna

11:15-11:45 Coffee

Decomposition and Carbon Cycling (Christoph Tebbe)

11:45 Paolo Nannipieri: The microbial origin of humic substances (Invited lecture)

12:10 Ellen Kandeler: Microbial Colonisation and Resource Partitioning in Agricultural Soils (Invited lecture)

12:35 Björn Lindahl: Ectomycorrhizal fungi drive humus decomposition in boreal forest (Invited lecture)

13:00-14:30 Lunch

Decomposition and Carbon Cycling (Lynne Boddy)

14:30 Rubén López-Mondéjar: What do they eat? Exploring the substrate-specificity of microbial communities in the decomposition of C sources in a forest soil

14:45 Annemieke van der Wal: Similar and contrasting patterns of natural fungal community assembly during initial decay of coniferous and broadleaf tree logs: an experimental common garden approach

15:00 Alexander Guhr: Redistribution of soil water by saprotrophic fungi enhances carbon mineralization

15:15 Ryan Williams: Elucidating microbial drivers of decomposition and the carbon cycle through a co-occurrence framework

15:30 Anders Tunlid: Spectroscopy and transcriptomics provide novel insights into soil organic matter decomposition mechanisms in ectomycorrhizal fungi

15:45 Pankaj Trivedi: Microbial regulation of carbon cycle: evidence from gene-enzyme relationship

16:00 Edith Hammer: Mycorrhizas: Pipeline or Director of Belowground C Fluxes?

16:15-16:45 Coffee

Interactions among Micro- and Macroorganisms I (Lynne Boddy)

16:45 Angela Sessitsch: The Hidden World Within Plants: Ecological Considerations and Functioning of Microbial Endophytes (Invited lecture)

17:10 Jan Frouz: The effect of soil fauna in decomposition activity of soil microflora (Invited lecture)

17:35 Julia Stevens: Recruitment of a beneficial rhizosphere community by the common dandelion (Taraxacum officinale) from different soil types

17:50 Max-Bernhard Ballhausen: The sapro-rhizosphere concept: Bacteria as secondary consumers of plant-derived carbon

18:05-19:45 **Poster session I** (all posters with ODD numbers)

TUESDAY, 1 DECEMBER 2015

Biodiversity and Functioning of Forest Soils (Jan Dirk van Elsas)

8:45 Petr Baldrian: Forest microbiome - diversity, functioning and dynamics (Invited lecture)

9:10 Lynne Boddy: Giants of the soil microbial world: foraging cord-forming fungi (Invited lecture)

9:35 Martin Hartmann: A decade of irrigation transforms the soil microbiome of a semi-arid pine forest

9:50 Christina Hazard: Genotypic Diversity Matters: Examining the diversity-ecosystem function relationship with ectomycorrhizal fungi

10:05 Fabian Bergkemper: Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems

10:20-10:50 Coffee

10:50 David Myrold: Microbial Community Response to Timber Harvest (Invited lecture)

11:15 Leho Tedersoo: Tree diversity and sampling effects on soil fungi, protists and meiofauna as revealed by multiplex ITS metabarcoding (Invited lecture)

11:40 Hui Sun: Microbial community shifts in structure and potential function across a boreal forest fire chronosequence

11:55 Eva Weber: Unravelling the ecological function of abundant but uncultivated Thaumarchaeota in acidic forest soils

12:10 Katerina Soldanova: Can we keep using soil ribosomal RNA as indicator of microbial activity?

12:25-14:00 Lunch

Soil Biogeochemistry and Nutrient Cycling (Paolo Nannipieri)

14:00 James Prosser: Bacterial ammonia oxidisers vs. archaeal ammonia oxidisers: who wins in soil, when and why? (Invited lecture)

14:25 Søren Sørensen: The communal gene pool in soil (Invited lecture)

14:50 Sara Hallin: Niche partitioning among N_2O reducing microorganisms and their importance as N_2O sinks

15:05 Dagmar Woebken: Combining stable isotope labeling experiments and single-cell analysis techniques to detect active microorganisms in soil

15:20 Constance Roco: The trajectories of denitrifier structure and function demonstrate remarkable differences in soil with a legacy of anoxic spells versus constant oxic conditions

15:35-16:15 Coffee

16:15 Jan Dirk van Elsas: The soil fungal-bacterial interactome - Mechanisms of interaction, with special emphasis on *Burkholderia terrae* (Invited lecture)

16:40 Jan Jansa: Arbuscular mycorrhizal fungi proliferate in patches of soil enriched with N-containing organic compounds

16:55 Marie Spohn: Do microbial carbon use efficiency (CUE) and the mean residence time (MRT) of microbial biomass depend on soil stoichiometry?

17:10 Natalie Lim: Regulation of nitrite concentrations in acidic and neutral pH soils by a combination of chemistry and complex bacterial community regulation: A study of kinetics and transcriptomics

17:25 Gu Feng: AM fungal hyphae exudates can prime a bacterium mediated phytate mineralization in hyphosphere

17:40 Joana Falcao Salles: Alien escape: impacts of bacterial invasions on soil microbial communities

18:00-19:40 **Poster session II** (all posters with EVEN numbers)

WEDNESDAY, 2 DECEMBER 2015

Biodiversity and Functioning of Agricultural Soils (Jan Frouz)

8:45 Christoph Tebbe: Microbiology of soil primary organo-mineral complexes and particulate organic matter (Invited lecture)

9:10 George Kowalchuk: Links between patterns of soil microbial diversity and sustainable soils (Invited lecture)

9:35 Michael Bonkowski: The diversity and functions of protists in soil: problems and progress (Invited lecture)

10:00 Dror Minz: What does the microbiome tell us about life in the plant root zone?

10:15 Wietse de Boer: Soil Volatile Organic Compounds: Microbial Competition Tools with High Potential for Control of Root-Infecting Pathogens

10:30 Germán Bonilla-Rosso: Evolution and Distribution Patterns of Nitrite Reductase (*nirK*/*nirS*) in Soil Metagenomes suggest Functional Differences Between Lineages

10:45 Frances Jones: The diversity of free-living, non-diazotrophic *Bradyrhizobium* from contrasting soils

11:00-11:30 Coffee

11:30 Zhong Wei: Biodiversity of synthetic microbial communities determines disease suppression

11:45 Gera Van Os: An indicator for disease suppression: linking soil chemistry to microbiology using dissolved organic carbon fractionation

12:00 Ameni Bahroun: Protozoa induce soil suppressiveness against *Fusarium* wilt

Interactions among Micro- and Macroorganisms (Jan Frouz)

12:15 Marc-André Selosse: Life strategy and life cycle of *Tuber melanosporum*: a pioneer hermaphrodite with high spore bank and functional dioecism (Invited lecture)

12:40 Mari Moora: Arbuscular mycorrhizal fungal communities: global and local patterns and their potential drivers (Invited lecture)

13:05 Aurelie Deveau: From mutualism to antagonism: iron acquisition during soil microbial interactions

13:20 David García de León Hernández: Can arbuscular mycorrhizal fungi drive vascular plant secondary succession in alvar grasslands?

13:35-15:00 Lunch

Trainbiodiverse – Exploring Soil Biodiversity across Europe (Søren Sørensen)

15:00 Søren Sørensen: Introduction to TRAINBIODIVERSE

15:12 Anne Schöler: DNA extraction methods have little impact on microbial community composition as assessed by amplicon sequencing

15:24 Barbara Bahnmann: Fungal communities across a mixed temperate forest: Are local site properties the most influential or does whose your neighbour matter?

15:36 Claudia De La Cruz Perera: Plasmid community adaptation in long-term copper contaminated soil as revealed by a comparative mobilome approach

15:48 Valentina Imparato: Dynamics of microbial communities in pre-exposed and pristine soils in response to high concentration of biochar

16:00 Inês Nunes: Coping with copper: Soil active bacterial communities following 100 years of exposure

16:12 Samuel Jehan Auguste Jacquinod: TBD

16:24 Irshad Ul Haq: Motility, stress responses and nutrients acquisition revealed by transcriptional profiling of *Burkholderia terrae* upon confrontation with a fungal host

16:36 Jean-Sebastien Beaulne: Large Scale Spatial Analysis of Bacterial Communities in Lake Sediments, the Role of Physico-Chemical Parameters, Spatial Distance, Land Cover and Tropical Storms

16:48 João Raimundo: Disentangling fine soil fauna-microbial interactions in mediating key soil processes under different land-use intensity systems and climate change scenarios

17:00-17:30 Coffee

17:30 Laura Sanguino : Using CRISPRs to learn about virus-host interactions in the environment

17:42 Maria de Vries: Phylogenetic and taxonomic diversity of glycoside hydrolase family 5 and 48-cellulase genes in agricultural soil

17:54 Salvador Lladó: Are the most abundant bacteria real key players in forest soil processes? A multi-omics approach

18:06 Shamina Pathan: Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in coniferous forest soil

18:18 Stephanie Jurburg: Autogenic succession in the soil microbial community

18:30 Susana Santos: Effects of land use on soil ciliate diversity

18:42 Sara Gallego: TBD

18:54 Divyashri Baraniya: Proteolytic soil communities and protease activity in rhizosphere of maize plants with different Nitrogen Uitilizing Efficiencies(NUE)

19:15-22:15 Conference dinner

THURSDAY, 3 DECEMBER 2015

Microbes in the Changing Environment (George Kowalchuk)

8:45 Erland Bååth: Bacterial growth responses to drying/rewetting and freezing/thawing – a tale of two patterns (Invited lecture)

9:10 Christa Schleper: Zooming in on the Functional Heterogeneity of Ammonia Oxidizing Archaea in Arctic Soils (Invited lecture)

9:35 Kornelia Smalla: Plasmid-mediated adaptation of soil bacteria to pollutants (Invited lecture)

10:00 Johannes Rousk: A case-study for traits-based theory and prediction in microbial ecology: colonisation of sterilised soils across a pH gradient

10:15 Tim Urich: Metabolic and trophic interactions modulate methane production by arctic peat microbiota in response to warming

10:30 Christian Poll: Impact of climate change on carbon cycling and soil microorganisms in an arable ecosystem

10:45 Marc Buée: Ectomycorrhizal and non-symbiotic fungi respond differentially to climatic parameters: what is the link with host susceptibility to climate change?

11:00-11:30 Coffee

11:30 Jiří Bárta: Vulnerability of cryoturbated carbon to climate change

11:45 Belinda Ferrari: Metagenomic insights into microbes living in the cold, extreme polar desert soils of Eastern Antarctica

12:00 Mark Anthony: Plant invasion (garlic mustard; Alliaria petiolata) alters fungal community composition, increases fungal diversity, and shifts dominant fungal trophic strategy

Archaeomicrobiology, Paleomicrobiology and Microbial Forensics (George Kowalchuk)

12:15 Bruce Budowle: Maturation of the Field of Microbial Forensics (Invited lecture)

12:40 Jeremy Austin: Predicting the origin of soil evidence: high throughput eukaryote sequencing and MIR spectroscopy applied to a crime scene scenario (Invited lecture)

13:05 Sandrine Demaneche: Microbial Soil Community Analyses for Forensic Science - Application to a Blind Test

13:20-14:00 Conference closing

LIST OF POSTERS

Decomposition and Carbon Cycling

90. Tobias Arnstadt - Log decay of *Fagus sylvatica* in temperate forests and the significance of lignin modifying enzymes for the degradation process
91. Chris Bamminger - Divergent effects of pyrochar and hydrochar on greenhouse gas emissions and microbial abundances in an arable soil
92. Andrea Burešová - Composition and activity of microbial community during decomposition of plant litter on two contrasting localities
93. Juliana Conceição - The management system can influence the physiological function and social interaction of phosphate solubilizing bacteria isolated rhizosphere of *Carica papaya* L.
94. Ivana Eichlerová - Decomposition traits and enzyme production of saprotrophic fungi are shaped by the combination of their ecophysiology and taxonomy
95. Lia del Pilar Fernández - Soil bacterial diversity from different animal settlements in maritime Antarctica
96. Damien Finn - Carbon and nitrogen co-metabolism and microbial nitrogen-mining both determine the extent of plant material decomposition in four Australian pasture soils.
97. Dimitrios Floudas - Evolutionary aspects of atromentin synthesis genes in Agaricomycetes
98. Diana Navrátilová - Spatial heterogeneity of decomposition and fungal community composition within single *Quercus petraea* leaves
99. Kevin Geyer - A comparison of methods for measuring the efficiency of microbial metabolism
100. Ton Gorissen - Uniformly ^{13}C-Labelled Biomass Tracers - Advances in ^{13}C-Techniques tracing changes in soil microbial processes and populations
101. Petra Havlíčková - Effects of plants on the structure, function and diversity of bacterial communities
102. Jussi Heinonsalo - Evidences on the ability of mycorrhizal genus *Piloderma* to use organic nitrogen and deliver it to Scots pine
103. Vincent Herve - Ecology and diversity of oxalotrophic bacteria - an in silico analysis
104. Björn Hoppe - Fungal functional diversity and enzyme activity patterns in decaying logs of 13 temperate tree species in an in situ decomposition experiment
105. Aicha Asma Houfani - Enzyme activities of aerobic (hemi)cellulolytic bacteria isolated from Algerian soils and compost
106. Dominika Chmolowska - Cellulose was decomposed faster in fallow soil than in meadow soil because of a quicker start of the process
107. Sarah Johnston - Fungus-Bacteria Interactions in Decomposing Wood
108. Grit Kabiersch - Detection of organotin compounds and degradation by litter-decomposing fungi
109. Katharina Keiblinger - Efficacy of biochar and compost on remediation of copper contamination in vineyard soils - effects on soil microbiology
110. Harald Kellner - Fungal research on an artificial deadwood decomposition experiment in the German Biodiversity Exploratories

111. Jaroslav Kukla - The influence of traditional agriculture on soil organic matter in tropical ecosystems of Papua New Guinea
112. Iuliia Kyiashchenko - The effect of soil fertility on fungal communities, enzyme activities and soil carbon dynamics in unmanaged forests
113. Sabrina Leonhardt - Fungal extracellular enzyme activity and biomass in coarse woody debris of 13 tree species in the early phase of decomposition.
114. Katya Litova - Studies on biodegradation of naphthalene and anthracene by *Aspergillus glaucus* strain isolated from Antarctic soil
115. Ashish Malik - Microbial communities' fungal to bacterial dominance alters carbon cycling in soil
116. Tomas Martin-Bertelsen - Towards linking fungal genes to chemical spectra from soil organic matter using machine learning
117. Tijana Martinović - Structure of microbial communities in the environmentally exposed construction wood samples of different species
118. Itamar Melo - Isolation and Screening of Highly Cellulolytic *Trichoderma* spp. from the Amazon Rainforest
119. Sophie Mieszkin - Effect of wood extractives on wood-degrading microorganisms and importance of the ecological niche
120. Karolin Müller - Turnover of Microbial Carbon in the Detritusphere
121. Cesar Nicolas Cuevas - Organic N decomposition by fungal community under fertilized spruce forest
122. Naoise Nunan - Carbon dynamics in Amazonian podzols under climate change
123. Michiel Op De Beeck - Soil organic matter degradation by ectomycorrhizal fungi
124. Tim Philpott - Fungal decomposition of fine roots in response to variable retention silviculture
125. Sebastian Preusser - Reciprocal Soil Transfer Experiments Improve the Understanding of Biological Regulation of Subsoil C-cycling
126. Salvador Rodríguez Zaragoza - Recovering of soil protozoan trophic groups after a strong pulse of hydrocarbon contamination
127. Mikhail Semenov - DNA-based determination of soil microbial biomass carbon under conditions of restricted applicability of substrate-induced respiration and fumigation-extraction
128. Sarker Mohammad Shakil - Characterization of Fe^{3+} reductants secreted by the closely related ectomycorrhizal fungus Paxillus involutus and the saprotrophic fungus *Hydnomerulius pinastri* during Fenton-based decomposition of organic matter
129. Ana Margarida Soares - Bridging the priming effect into aquatic systems - Primary producer-C stimulates the fungal decomposition of submerged litter
130. Florian Strasser - Influences of carbon substrates and nitrogen availability on microbial-mediated cellulose degradation in an Austrian beech forest soil
131. Lucie Štercová - Fungal biodiversity of wood decomposing species in national nature reservation of Salajka
132. Rodrigo Taketani - Litter decomposition in mangroves - the role of microbes revealed by DNA and mRNA sequencing
133. Aloysius Teo - Using teabags to estimate decomposition rates across primary and secondary tropical forests, and investigating the functional role of termites
134. Vojtěch Tláskal - Linking deadwood age with inhabiting bacterial community

135. Tomas Větrovský - Sequence processing fast and easy - SEED a GUI based user friendly sequence editor and pipeline for high-throughput amplicon processing
136. Alexandra Wolf - Agro-ecosystem type and soil aggregate size impact soil carbon dynamics
137. Ilya Yevdokimov - Microbial immobilization and incorporation into DNA of inorganic ^{33}P-labelled phosphorus
138. Lucia Žifčáková - Correlation of lignocellulolytic genes expression and their activity in ME fungal cultures

Soil Biogeochemistry and Nutrient Cycling

139. Roey Angel - Optimizing the toolbox to investigate free-living diazotrophs in soil - from bulk measurements to single-cell analysis.
140. Doreen Babin - Effect of phenanthrene on the release of mobile organic matter and the bacterial community structure in soil
141. Paul Bodelier - Unexpected stimulation of soil methane uptake by bio-based residue application - An emerging property of agricultural soils offsetting greenhouse gas balance
142. Runa Boeddinghaus - Land-use intensity and physico-chemical soil properties have distinct effects on microbial communities and enzyme activities of grassland soils
143. Ivano Brunner - Shifts of C and N isotopes in fruiting bodies of fungi after 12 years of irrigation of a semi-arid pine forest
144. Elisa Catão - Ammonia oxidizers in a non-nitrifying Brazilian savannah soil
145. Maria Cucu - Different agricultural practices drive aerobic and anaerobic ammonia oxidisers niche segregation in a temperate paddy soil
146. Andreas Demey - Impact of bioavailable phosphorus on plant and soil microbial communities in grassland under restoration management
147. Stefan Forstner - How does long-term nitrogen input influence stoichiometric relationships between soil microbes and their resources?
148. Bo Fu - The effect of temperature on the carbon isotope value of acetate in Philippine rice field soil
149. Ahlam Hamim - Phosphate solubilizing microorganisms isolated from root and rhizosphere soil of ericaceous shrubs in the north of Morocco.
150. Christine Heuck - Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus
151. Jiezhong Chen - The Expression Analysis of Plasma Membrane Aquaporin Gene EjPIP2 in Eriobotrya japonica After AM Fungi Inoculation
152. María Irisarri - Do soil type, rice cultivar and water management affect the bacterial denitrifying community of a paddy soil?
153. Sheku Kanu - Interactive effects of Bacillus subtilis and seaweed (kelpak) on the growth, metabolites and yield of potato (Solanum tuberusom L.) under glasshouse conditions
154. Késia Lourenço - Effected of regular or concentrate vinasse on greenhouse gases emissions from soil with sugarcane

155. Sven Marhan - Nutrient limitation of soil microorganisms - effects of grassland land-use intensity
156. Dora Neina - Restoring the functional integrity of a Technosol with native organic materials
157. Maximilian Nepel - Identifying potential key players of N2 fixation in European biological soil crusts
158. Laurent Philippot - Recently identified microbial guild mediates soil N2O sink capacity
159. Frank Rasche - Lasting influence of biochemically contrasting organic inputs on abundance and community structure of total and proteolytic bacteria in tropical soils
160. Karolina Tahovská - Microbial activity in the context of acid deposition – field manipulations with sulphur and/or nitrogen inputs to the forest soils
161. Irina Tanuwidjaja - Influence of different clay minerals on the microbiome of soils and its functionality in simplified artificial systems
162. Cecile Thion - Predicting temporal and spatial variations in bacterial phylogenetic and phenotypic community structure in glacier forefield chronosequences
163. Yang Zhou - The functional profiles of soil microbial communities are determined by soil chemical properties but not community composition

Biodiversity and Functioning of Agricultural Soils

164. Pilar Andrés - Effect of biochar application to soil on soil microbial communities structure and feeding habits - a field study in Mediterranean soils
165. Olubukola Babalola - Metal tolerant, plant growth promoting soil bacteria protected plants against the toxic effects of heavy metals (Cd, Cr, and Ni)
166. Guillaume BAY - Effects of cropping system, depth, and sampling time on soil microbial communities
167. Andrew Bissett - Effects of temporal pH shifts on ammonia oxidiser community structure and function
168. Ian Clark - Response of Bacterial and Archaeal nitrifying populations to changing landscapes
169. Benjamin Costerousse - Characterization of the bacterial processes responsible for zinc solubilization in wheat rhizosphere
170. Florine Degrune - The influence of soil tillage on microbial communities changes along the soil profile
171. Anderson Ferreira – Soil bacterial community under integrative production system at biomes savanna and Amazon
172. Davide Francioli - Rhizosphere microbiome, plant community and soil nutrient availability - a new approach to survey the bacterial assemblage in soil
173. Mercedes García Sánchez - Digestate and fly ash applications in agricultural soils - impact in the biomass and biodiversity of fungal communities.
174. Aurelia Gebala - Does Land-Use Intensity Influence Microbial Resource Partitioning and Microbial Colonization Strategies of Organo-Mineral Complexes in Grassland Soils?
175. Mariangela Girlanda - Plant genotype control over the recruitment of the tomato fungal microbiota

176. Daniel Graf - Community assembly processes of N2O reducing prokaryotes in the rhizosphere- effect of edaphic factors and plant species
177. Yian Gu - Pathogen-induced shifts in exudation alter the rhizosphere microbiome
178. Moritz Hallama - Soil Microbial Phosphorus Dynamics are Affected by Cover Crops and Minimum Tillage
179. Penny Hirsch - Abundance and activity of soil microbial communities revealed by metagenomics and metatranscriptomics
180. Anna-Sofia Hug - Soil microbial diversity patterns at Sites of the Swiss Soil Monitoring Network
181. Christopher Jones - Field-scale spatial variation in co-occurrence patterns of ammonia and nitrite oxidizing communities.
182. Milko Jorquera - Exploring rhizobacterial community composition associated with plants grown in Chilean extreme environments using 16S rRNA-based molecular approaches
183. Hans-Martin Krause - Influence of soil management history on microbial N2O production and reduction
184. Iva Krizkova-Kudlikova - Characterization of Actinomycetes Antagonistic to Streptomyces spp.
185. Martin Krsek - Bead-beating and isolation of environmental nucleic acids
186. Volery Lara - Effects of management on soil microorganism communities in Swiss vineyards
187. Guillaume Lentendu - Alfalfa root symbionts under soil nutrient pressure - cooperation or competition?
188. Hongwei Liu - Activation of salicylic acid defence signalling pathway reduced Archaea abundance and genes involved in nitrogen and carbon cycling in wheat rhizosphere
189. Pawel Lycus - Newly isolated denitrifiers from low and high pH soil show little correlation between genotype and phenotype
190. Jarmila Makovníková – The potential of agroecosystem services in relation to land use and biodiversity
191. Lokeshwaran Manoharan - Enzymes related to organic matter degradation and agricultural management
192. Shinsuke Mori - Changes in the oxidation-reduction potential and in bacterial profiles in the soil around direct-seeded rice under submerged conditions
193. Esther Muema - Biochemically contrasting organic inputs combined with mineral nitrogen fertilizer shape the temporal variation of ammonia-oxidizing prokaryotic communities in an agricultural soil
194. Mary Musyoki - Soil type, season and crop growth stage exert a stronger effect on rhizosphere microbial dynamics than the fungal biocontrol agent Fusarium oxysporum f.sp. strigae
195. Lidia Nicola - Fumigation with Dazomet modifies soil bacterial and fungal communities in soil of apple orchards affected by Specific Replant Disease
196. Ansa Palojärvi - Improved general plant pathogen suppressiveness by agricultural management practices
197. Martina Putz - Long-term nitrogen fertilization affects microbial communities regulating N2O emissions in arable soils
198. Vivian Rincon Florez - Impact of strategic tillage on nitrogen cycle genes (amoA and nifH) in no-till systems in Queensland, Australia

199. Jenna Ross - Isolation of a novel ammonia oxidising archaeon, representative of the Nitrososphaera 'sister' lineage
200. Suikinai Nobre Santos - Annotation of gene cluster Involved phenazine biosynthesis in Streptomyces CMAA 1322 also too structural elucidation of 1,6 dimethoxyphenazine.
201. Rumakanta Sapkota - Soil oomycete community structure association with cavity spot disease of carrot
202. Alise Senberga - Evaluation of Effectiveness of Rhizobia and Plant Interaction in Different Soil Types
203. Abhi Shah – Microbial activity along a continuous subsurface core from an agriculture field at the estuarine region of Mahi river: correlation with sediment characteristics
204. Hannes Schmidt - Diversity and spatial distribution of diazotrophs associated with micro-environments of wetland rice
205. Elvira Schnyder - Methanotroph diversity increases methane oxidation
206. Susanne Schreiter - Roots of decline? – The SARISA project
207. Magdalena Steiner - Microbial diversity and ecosystem functioning in vineyards
208. Nicolas Theodorakopoulos - Microorganism's enzymes implication in nitrous oxide emissions in a natural agricultural field at a fine time scale study
209. Maaike van Agtmaal - Exploring the phytopathogenic seedbank of agricultural soils - diversity of soil borne plant pathogens in relation to edaphic properties and the soil microbial community
210. Wu Xiong - Effects of black pepper-vanilla rotation on vanilla rhizosphere fungal communities in relation to Fusarium wilt disease
211. Tianjie Yang - Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health
212. Qing Yao - The functional profiles of soil microbial communities are determined by soil chemical properties but not community composition
213. Judith Zimmermann - The biocontrol agent Fusarium oxysporum f.sp. strigae – its detection and effects on beneficial indigenous microorganisms in a maize rhizosphere
214. Josephine Zimudzi - The microbial communities associated with potato rhizosphere under different seasonal conditions in South Africa

Biodiversity and Functioning of Forest Soils

215. Sarah Addison - Soil microbes and their importance in shaping our forests using qPCR
216. Toke Bang-Andreasen - Responses in Active Microbial Communities and Expression of Important Functional Genes in Forest and Agricultural Field Soil after Wood Ash Addition Revealed by Metatranscriptomics
217. Tommaso Bardelli - Complex effects of altitude and exposure on microbial communities in (sub)alpine soils
218. Felipe Bastida - Landscape proteogenomics - explaining the functional-phylogenetic relationships of microbial communities by gradients of organic C availability in soil
219. Garazi Benito Carnero - Tree species effect on soil microbial community
220. Monique Carnol - Carbon substrate utilization and microbial biomass in European forest soils are related to tree species diversity

221. Carles Castaño Soler - Drying treatment of soil samples affects DNA recovery but does not change the fungal community structure by metagenomic analysis
222. Carla Cruz-Paredes - Using fungal and bacterial growth to evaluate the effects of ash application on forest soils
223. Timo Domisch - Winter in a changing climate affecting the survival of Scots pine seedlings
224. Beat Frey - Pyrosequencing based assessment of bacterial and fungal community compositions in compacted and regenerated forest soils
225. Anna Frymark-Szymkowiak - Soil ß-Glucosidase activity under canopy of White Poplar in riparian forests.
226. Kezia Goldmann - Spatial variation of the fungal metagenome in temperate beech forests across Germany
227. Erika Gömöryová - Soil microbial community changes in the disturbed Norway spruce stands during a 10-years period
228. Sue Grayston - CH4 and N2O microbial communities respond to site preparation and fertilization in wet forests
229. Michal Choma - Recovery of ectomycorrhizal community of a boreal forest after three decades of N fertilisation
230. Leticia Izquierdo - A new promising molecular marker to study the functional diversity of fungal communities - the glycosyl hydrolase 63 gene
231. Veronika Jílková - Methane flux in wood ant (Formica polyctena) nests and the surrounding forest floor
232. Jan Kopecký - Metabolite profiles of soil actinobacteria follow their phylogeny and environmental factors at the isolation sites
233. Diego Leiva Cáceres - Relations between Peltigera lichen's derived factors and its associated bacterial communities
234. Sandrine Malchair - Spatial variability of soil microbial processes in a temperate mixed forest
235. Oscar Martínez - Fungal communities associated with rhizosphere of Nothofagus alpina from different volcanic ash-derived soils in southern Chile
236. David Myrold - Development and Decline of Microbial Communities Associated with Ectomycorrhizal Mats
237. Pascal Nassal - The importance of fungal-fungal and bacterial-fungal interactions for phosphorus dynamics in forest soils
238. Mike Ogden - Fine scale modification of soil physical properties by fungi - reinforcement and repellency in the hyphosphere
239. Jade O'Leary - Multi-dimensional mycelia interactions
240. Xavier Parlade – Dynamics of Boletus edulis extraradical soil mycelium and sporocarp production in managed forests
241. Taina Pennanen - Virus host switches between pathogenic, mycorrhizal and saprotrophic fungal species in a boreal forest
242. Camilla Pereira – Arbuscular mycorrhizal fungi in protected areas of northeastern Brazil
243. Diogo Pinho - A first look at the Quercus suber (cork oak) root microbiome - differences between healthy and declined trees
244. Flavia Pinzari - Overlap in the metabolic functions of cellulose-decomposing leaf litter fungi

245. Tereza Poláčková - Ecology of soil yeast communities in mixed temperate forest soils
246. Daria Rapoport - Isolation and cultivation of actinobacteria from acid soils with high occurrence of Trebon clade
247. Ana Rincón - Fire recurrence effects over the structure and activity of ectomycorrhizal fungal communities in Mediterranean pine forests
248. Alice Roy-Bolduc - High richness of ectomycorrhizal fungi and low host specificity in a coastal sand dune ecosystem
249. Minna Santalahti - Revealing sources of biological methane production in boreal upland forest
250. Outi-Maaria Sietiö - The effect of photosynthesis-derived C flow on the microbial community structure and enzymatic activities in boreal forest
251. José A. Siles - Archaeal, bacterial and fungal abundance and diversity along an altitudinal gradient in Alpine forest soils
252. Izabela Sondej - Impact of wild boar (Sus scrofa) rooting on the soil seed bank in Białowieża Forest.
253. Martina Šturšová - Spatial heterogeneity of mountainous soil is associated with high beta diversity of microbial community
254. Martina Vašutová - Mycorrhizal community structure across an alpine tree line ecotone
255. Christina Weißbecker - Patterns of soil fungal communities in subtropical Chinese forests in relation to plant diversity

Interactions among Micro- and Macroorganisms

256. Ina Alsina - The yield of onions and its quality depending on mycorrhiza inoculation
257. Lucas Braga – Earthworm-microbe interaction can be associated to less harsh conditions in green sugarcane systems
258. Ana Correia - Carbon source and availability influence the production of antimicrobial compounds
259. Alper Dede – Rhizosphere of olive tree: a source of plant growth promoting bacteria
260. Laila Dubova - The effects of michorrhyza fungy on the tomatoe plant water retention ability
261. Iva Cholakova - Characterization of plant-associated bacteria isolated from highly drought tolerant Pistacia therebinthus
262. Polina Ivanova - Antibiotic activity of actinobacteria associated with millipedes and earthworms
263. Xianwang Kong - Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on N2O emissions from clover residues and interaction with the earthworm Lumbricus terrestris
264. Tat'yana Kotova - Suppressive activity of the intestinal fluid of diplopods against yeasts
265. Ines Mandic Mulec - Kin discrimination between sympatric soil isolates of Bacillus subtilis
266. Lucas Mendes - The role of rhizosphere microbiome in soilborne fungal disease suppression in common bean
267. Anna Rawlings - Decay in the canopy

268. Ruth Schmidt - Microbial airborne talk - effect of fungal volatiles on bacteria
269. Ana Soares - Specificity and biotic local selection in Streptomyces interactions influences antimicrobial activity
270. Tamara Těšitelová - Two widespread green Neottia species (Orchidaceae) show mycorrhizal preference for Sebacinales in various habitats and ontogenetic stages
271. Sannakajsa Velmala - Function by form - a tentative insight to the link between growth and the diversity of ectomycorrhizal fungi
272. Zhihui Xu - Comparative proteomics analysis of Bacillus amyloliquefaciens SQR9 revealed the key proteins involved in in situ root colonization

Microbes in the Changing Environment

273. Abdulmajeed Al Khajeh - Fish emulsion as a food base for halophilic actinomycetes promoting growth of Salicornia bigelovii in a sandy soil in the United Arab Emirates
274. Rana Shahbaz Ali - Effects of substrate complexity and temperature on growth of different microbial groups
275. Sawa ARAI - Carbon-starvation in light induced tolerance to hyperosmotic stress in purple photosynthetic bacterium Rhodopseudomonas palustris
276. Andrea Borsodi - Microbial communities inhabiting the rhizosphere of halophyton plants living nearby Hungarian soda lakes
277. Tabitha Bucher - Disturbance of the bacterial cell wall specifically interferes with biofilm formation
278. Cristina Cruz - Alleviating the N limitation expands the possibilities for structuring soil bacterial communities - evidence based on the impacts of 5 years' manipulation of N dose and form in a Mediterranean ecosystem
279. Jonathan De Long - Soil microbial and nematode communities respond differently to warming and plant functional group removal across a post-fire boreal forest successional gradient
280. Teresa Dias - Impacts of N enrichment on Mediterranean biological soil crusts community and functions - the unseen evidence from soil pigments
281. Francisco Dini-Andreote - Unveiling the blueprint of marine-terrestrial transition in bacterial adaptation and evolution
282. Dana Elhottová - Bacterial antibiotic resistance heterogeneity in natural subterranean habitats
283. Huyuan Feng - Influences of Long-term Nitrogen Fertilization on Fungal Endophyte Community of Three Grasses in an Alpine Meadow
284. Nawras Ghanem - The transport of marine phages in soil as a tool of understanding the interaction of surface-subsurface events
285. Osnat Gillor - Hydration dynamics in desert soil mediate antagonism of actinobacteria
286. Sydney Glassman - Ectomycorrhizal fungal spore bank recovery after a severe forest fire: Some like it hot
287. Catarina Gouveia - Effect of Increased N availability on Ammonium oxidizing bacteria populations – A possible Bioindicator in Mediterranean ecosystem
288. Kelly Gravuer - Phylogenetic estimation of ecologically important traits illuminates microbial community responses to change in natural and agro-ecosystems

289. Stefan Green - Methodological Improvements to Amplicon-Based Surveys of Microbial Community Structure
290. Jeremiah Henning - Fungi on mountainsides - contrasting elevational and seasonal patterns among root-associated fungal groups.
291. Andreas Herrmann - Spatial and seasonal variability of the microbial community in forest fen soils on North-East-Germany
292. Jana Judova - The Contingency of some Biotic and Abiotic Parameters in Arable Land and Permanent Grasslands
293. Tatiana Khomutova – Characterization of microbial pool in sub-kurgan paleosols of different ages in desert-steppe zone in relation to the holocene dynamics of climate
294. Sara König - Hot spots and cold spots – modelling biodegradation dynamics under disturbance regimes
295. Ramóna Kovács - Characterization of mycorrhizophere in a Hungarian saline-sodic grassland
296. Anna Kuznetsova - The first study of actinomycetes complexes in Prietonie region soil
297. Xiaofei Lyu - Soil bacterial community along a successional series of tidal flats in the Yellow River Delta
298. Aurora MacRae-Crerar - Ecological determinants of soil bacterial community structure across multiple scales in a Mongolian global change experiment
299. Marta Misiak - Soil fungal responses to warming in polar regions
300. Luis Morgado - Compositional shifts in arctic ectomycorrhizal fungal community in response to long-term increased snow depth in Northern Alaska
301. Eric Morrison - Soil warming changes litter chemistry and fungal community composition but not decomposition rate
302. Sally Otto - Catch me if you can – The impact of mycelia-based dispersal on predator-prey interactions and biodegradation of soil contaminants
303. Prashant Pant - 16S rRNA gene family based microbial typing of rhizospheric communities of a native legume Alysicarpus vaginalis (L.), fam. Fabaceae.
304. Elizaveta Pershina - Looking for the core microbiome of the main types of soils in Russia
305. Kristin Rath - The influence of salinity on saprotrophic fungi and bacteria in soil
306. Klára Řeháková - Potential activity of microbial community in the Biological Soil Crusts
307. Nermina Saronjic - How do soil microbial communities react on droughts and heavy rainfall events?
308. Philipp-André Schmidt - Response of Soil Fungal Communities to Extended Drought
309. Frank Solano-Campos - Preliminary data of soil nematode communities along a rainfall gradient in Costa Rica.
310. Afnan Suleiman - Active microbial community resilience in disturbed soil with nitrogen source enrichment and nitrification inhibitor
311. Tibor Szili-Kovács – Genetic diversity and catabolic activity profiles of rhizosphere bacterial communities during dry and wet seasons in a solonchak grassland, Hungary
312. Alexandra Šimonovičová - Spatial distribution of microscopic fungi under old environmental burdens
313. Adam Šťovíček - Bacterial response to rainfall and draught cycles in desert soil
314. Daniela Trojan - Investigating the ecophysiology of the ubiquitous Acidobacteria in the dynamic soil environment
315. Tushar Yadav - Effect of Cosmetic Based Nanowaste on Sludge and Soil Microflora

316. Baogui Zhang - Response characteristics of soil microorganism to permafrost degradation in the upstream regions of the Shule river basin, Qinghai-Tibetan Plateau
317. Junling Zhang - Unexplored Biodiversity and Function of arbscular mycorrhizal fungi on the Tibetan Plateau

Microbial Life in Contaminated and Anthropogenic Soils

318. Valeria Ancona - Microbiological indicators to evaluate soil quality of degraded areas in Southern Italy after compost addition
319. Thomas Banitz - Spatial metrics indicate bacterial degradation benefits from mycelial networks
320. Angelantonio Calabrese - Use of molecular techniques to characterize the microbial communitiesfor soil ecology assessment in degraded sites.
321. Juan Campos - Soil dehydrogenase activity under the presence of some exobiotics. A toxicity index is proposed.
322. Stefano Covino - A pyrosequencing-based metagenomic study of microbial communities during co-composting of creosote-impregnated wood and green wastes
323. Sabrina Festa - Monitoring the impact of bioaugmentation with a PAH-degrading strain on different soil microbiomes using pyrosequencing
324. Alena Filipová - A novel bioaugmentation approach for PAH-degrading bacteria in soil: Adaptability as assessed by molecular biology techniques
325. Fanny Flores - Microbial activity of chromium polluted soil from Guanajuato México, during in situ biostimulation assay.
326. Ulrike Gerber - Interactions of natural occurring eukaryotic microorganisms with uranium(VI)
327. Paola Grenni - Effects of compost addition and Medicago sativa occurrence on PCB biodegradation in a historically contaminated soil
328. Anna Grobelak - The possible application of microorganisms in promoting plant growth and improving plant biomass in the phytoremediation of anthropogenic and contaminated soils
329. María Gutiérrez Núñez - Non-target effects of pesticides on the microbial activity in agricultural soil
330. Lenka Harantová - Factors influencing microbial community development during primary succession on spoil heaps after brown coal mining
331. Karin Hellauer - Sequential managed aquifer recharge leads to a high diverse microbial community resulting in a better attenuation of moderate degradable trace organic chemicals (TOrC)
332. Anne Houles - Ecological restoration of nickel mine sites in New Caledonia - Characterisation of ectomycorrhizal fungal community the key enabling the monitoring of facilitation process between plants.
333. Alica Chroňáková - Boreal acid sulphate soils – changes in bacterial communities along vertical profile and between total and active pools
334. Inge Jambon - Bioremediation of chlorendic acid, a highly chlorinated organic pollutant, by exploiting a fungal-bacteria consortium native to the contaminated field

335. Michal Kaminski - Omics approach in analysis of Pseudomonas mandelii ssp. capable of bioaccumulating hexachlorocyclohexane
336. Hülya Kaplan - Diversity of bacteria involved in 13C-labelled wheat root decomposition and efflux-mediated metal resistance in metal contaminated soils remediated with amendments
337. Pawel Krawczyk - Bioinformatic approach to analysis of plasmid pool in metagenomes from polluted soils
338. Jennifer Mesa Marin - Ecology of soil bacteria in bioremediation - indigenous plant growth promoting rhizobacteria in native Spartina maritima as a tool for the restoration of heavy metal polluted salt marshes
339. Zuzana Michalkova - Interactions of nano zerovalent iron with Acidithiobacillus ferooxidans – Implications for soil remediation
340. Annett Mikolasch - Oil-degrading bacteria isolated from the rhizosphere of plants growing in oil-contaminated soils from Kazakhstan
341. Irma Morelli - Pyrosequencing reveals bioaugmentation impact on the dynamics of bacterial community on phenanthrene-contaminated soil
342. Marta Moreno Valencia - Effect of olive and vine wood ashes on the dehydrogenase activity in a crop land.
343. Martina Plačková - Bacterial community characteristics under decades-lasting antibiotics selection pressures
344. Thomas Pommier - Response of soil microbial community to titanium dioxide nanoparticles - a cascading pitch on the nitrogen cycle
345. Pavla Průchová - Composition and activity of microbial communities in soil contaminated by heavy metals
346. Rashmi Saikia - Influence of sources of carbon in growth media on the yield of biosurfactant by the microbe isolated from crude oil contaminated soil
347. Hokyung Song - Abandoned tropical tin mine site shows chnges in microbial community with restoration
348. Gangavarapu Subrahmanyam - Abundance and diversity of ammonia oxidizing archaea and bacteria in long-term industrial effluent polluted soils, Gujarat, Western India
349. Karel Švec - Composition of fungal and bacterial communities in mercury polluted areas
350. María Touceda-González - Molecular characterization of the rhizobacterial communities of two Ni-hiperaccumulating subspecies of Alyssum serpyllifolium endemic to the Iberian Peninsula.
351. Gustavo Valdecantos - Biotic and abiotic factors affect the colonization and the dynamics of bacterial community assemblage in irradiated soil microcosms
352. María Vásquez Murrieta – Metal tolerance and biosorption potential of endophytic fungi isolated from Bahia absinthifolia
353. Carl-Eric Wegner - Persisting in slag – insights into aluminium resistance from early industrial mineral leaching
354. Franco Widmer - Effects of different nanoparticles on soil microbial community structures and plant-microbe interactions
355. Yucheng Wu - Responses of Thaumarchaeotal community in agricultural soils to acidification and polycyclic aromatic hydrocarbons contamination

356. Lijuan Yan - Comparative phylogenetic analysis of bacterial community dynamics during multi-year bioremediation of oil-contaminated soil in a boreal climate

Archaeomicrobiology, Paleomicrobiology and Microbial Forensics

357. Cecile Gubry-Rangin - Molecular adaptation of the ammonia monooxygenase amoA gene during the ancient and rapid diversification of terrestrial Thaumarchaeota
358. Eline van Asperen - Establishing dung fungal spores as a proxy for herbivore abundance - an experimental approach

Function First: New Ways to Study Soil Microbes on a Single Cell Level

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Soils harbor a tremendous microbial diversity and the functional traits of these bacterial and archaeal inhabitants are essential for the health of all terrestrial ecosystems and for the services they provide for humans. Application of metagenomic techniques has significantly advanced our understanding of the genetic potential of soil microorganisms, but nevertheless for many important processes in soils the microbial key players have not yet been identified and characterized. In this talk, I will illustrate using nitrifying microbes as an example that novel and totally unexpected functions can be catalyzed by apparently functionally constrained microorganisms and that it is impossible to infer from genomic data alone what a microbe is actually doing in the environment. To overcome this major limitation, I will present a new approach that allows microbial ecologists to specifically identify all active microbes in a soil sample under defined conditions (e.g. in the presence or absence of an added substrate) by combining heavy water incubations with Raman microspectroscopy, NanoSIMS and fluorescence in situ hybridization (FISH). Finally, a new methodological pipeline will be presented that allows to sort single microbial cells with a specific function from soil samples for subsequent genomic characterization or cultivation.

Unearthing the Roots of Fungal Symbioses

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Forest health, productivity and sustainability depend on above- and below-ground microbial associations to exchange nutrients, recycle carbon, and sustain diseases and harsh environmental conditions. Fungi are often described as either saprotrophs, which degrade complex organic substrates, or biotrophs, which obtain carbon compounds from living hosts. Among the latter, ectomycorrhizal (ECM) fungi provide crucial ecological services in interacting with most forest trees. They are portrayed as mutualists trading plant host photoassimilates for nutrients and having limited capacity to decompose soil lignocellulose. An improved understanding of the role of ECM fungi and their evolutionary adaptive history in the face of changing environmental conditions will create tools to predict how they are likely to adapt to future climate change. A major goal of mycorrhizal studies is also to define the symbiosis in molecular terms, i.e. to identify the 'symbiosis genes' that encode the molecules that mediate and regulate symbiosis development and the coordinated symbiotic metabolic pathways.
To identify the genetic innovations that led to convergent evolution of the mycorrhizal lifestyle from ancestral saprotrophic species, we have conducted the first broad, comparative phylogenomic analysis of mycorrhizal fungi, drawing on 50 genomes from ECM, orchid (ORM) and ericoid (ERM) fungi. The analyses of these genomes suggested that mycorrhizal symbioses evolved from ecologically diverse decayer precursors and radiated in parallel, following the origins of their host- plant lineages. Polyphyletic evolution of the ECM lifestyle is marked not only by convergent losses of different components of the ancestral saprotrophic apparatus but also by rapid genetic turnover in symbiosis-induced genes, some of which may reflect lineage-specific functional innovations, such as effector-like mycorrhiza-induced small secreted proteins (MiSSPs). In contrast, ERM and ORM fungi retained an extensive decay apparatus that is probably exploited indirectly by the plant for carbohydrate supply, thus explaining their known saprotrophic ability. The available genome sequences of mycorrhizal fungi will represent foundational information for understanding symbiosis development and func- tioning. These resources will facilitate field studies aiming to predict responses of mycorrhizal communities to environmental shifts, such as altered forest-management practices and climate change.

Reconstruction of microbial nutrient cycles in soil using metagenomic approaches

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Soil microbiomes are very important for soil quality and environmental services, like the provision of clean water, a sustainable agricultural production or the recultivation of soils. In the past, studies often focused on the function or diversity of a rather restricted group of microbes using PCR based approaches. Consequently, a lot of important processes or microbial taxa might have been overlooked, which are not targeted with the respective primer systems. Thus it is assumed that more than 90 % of the microflora and its genetic potential from the environment have not been described so far.
However, the progress in molecular technologies, especially in next and second generation sequencing techniques, allows an unbiased view on the community structure and function of ecosystems. So today we are able not only to assess single pathways which are catalyzed by microbes in various environments, but to reconstruct whole nutrient cycles and to identify the contributing bacteria fungi and archaea by using metagenomic approaches that are independent from PCR. However the restricted sequencing depth (one gram of soil may harbor more than 5 Tbp of information and most approaches published so far do not go beyond sequencing 50 - 100 Gbp) allows only semi-quantitative predictions, with have to be confirmed using classical more targeted approaches derived from molecular microbial ecology like qPCR or microarray technologies. We have named this as second generation full cycle approach.
The presentation will show some examples from different soil ecosystems, where these approaches have been successfully used to reconstruct nutrient cycles like N und P or to identify major players involved in carbon turnover.
However there are still a number of drawbacks that need to be taken into account, which includes the frustrating high number of still unknown sequences (up to 30 % depending on the type of filtering used), which can be neither assigned to a known phyla nor to a so far identified functional trait. Furthermore the high diversity of microbes in a given environment makes an assembly of larger contigs and thus the description of induction and repression mechanisms of particular genes impossible. Thus future approaches should include also the definition of new enrichment and isolation strategies based on the obtained metagenomic and transcriptomic data (third generation full cycle approach).

Microbial Social Networks in Contaminated Soils

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Microbial response to the arrival of significant concentrations of chemical compounds, whether xenobiotic or not, leads to a shift in the structure of the microbial community. The categories of microbial response include 1) those that can use the compound(s) for energy and/or biomass, 2) those that benefit indirectly from those (1) that use the compound(s) directly, and 3) those that do not benefit (either by being outcompeted or by toxic or inhibitory effects). In addition, microorganisms might benefit from one compound and produce metabolites that help others benefit from other compounds. These types of relationships can continued to be described with ever increasing complexity. As a first step in unraveling these multiple relationships in soil microbial communities, we have tried to determine the microorganisms and ecosystem functions that are relatively important (defined later) to different microorganisms that are critical to the degradation of specific compounds considered to be pollutants. This initial step does not yet test any hypotheses concerning the type of relationship between the different microorganisms, although functional signals sometimes are consistent with these hypotheses.

Our approach was to determine the covariance of microorganisms (16S rRNA gene sequencing or phylochips) and functions (high throughput sequencing of environmental DNA) in a range of soils that have undergone contamination. In terms of bioinformatics, we have constructed social networks not unlike that used by software companies trying to determine who you know and who you might like. The robustness of this approach depends on the number of situations used in the calculations as well as the specificity of different relationships. When applied to the anaerobic degradation of chlorinated compounds, the known dechlorinators had hydrogen and acetate producers on their social network page. They also had some specific electron transfer functions on their pages. We hope that this approach will aid in deciphering the different microbial requirements for a healthy contaminant degrading soil.

Insights into ecology during microbial bioremediation as assessed through advanced techniques – case studies

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Various bioremediation techniques have been introduced into practice long time ago for the reclamation of contaminated sites. These methods rely on either the applications of allochthonous microorganisms (bioaugmentation) or on the biostimulation of autochthonous populations to achieve the removal of relevant pollutants. Modern cultivation-independent techniques allow to describe in-depth the composition of natural populations as well as to study interactions or cooperation within members of the microbial community upon remediation intervention. This contribution presents two different case studies of remediation, in which the use of a next generation sequencing technique (454-pyrosequencing) and phospholipids fatty acid (PLFA) analysis, together with a detail physico-chemical analysis of the treated sites, enabled to gain insights into the mechanisms and processes ongoing during the remediation.

The first case study represents a pilot remediation test combining two Cr(VI) geofixation methods – chemical reduction by nanoscale zero-valent iron (nZVI) and subsequent biotic reduction supported by whey. The results indicated that nZVI oxidized to Fe(III) during the abiotic phase was microbially reduced back to Fe(II) and acted as a reducing agent for Cr(VI) even when the microbial density was already low due to the consumed substrate. Community analysis with pyrosequencing of the 16S rRNA genes further confirmed that the process was mediated mainly by iron-reducing and sulfate-reducing bacteria.

The second study was aimed at complex characterization of soil samples from a site historically contaminated with polychlorinated biphenyls (PCB). PCB degradation intermediates, namely chlorobenzoic acids, were detected in all the soil samples, suggesting the occurrence of microbial transformation processes over time. One of the studied samples was used in a an experiment where two white-rots Pleurotus ostreatus and Irpex lacteus were applied in order to decompose PCBs. Especially P. ostreatus exhibited significant degradation abilities and the original contamination dropped down to about 40% within 24 weeks. Further analyses using PLFA analysis and pyrosequencing suggest that the fungi use different strategy toward the autochthonous microflora and the results indicate possible cooperation.

The results form both the studies emphasize a need to study further ecology of bioremediation and to clarify mechanisms and processes involved in the technological applications.

Degradation of ^{13}C-labelled pyrene in soil-compost mixtures and farmyard fertilized soil – turnover mass balances and community analyses

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Polycyclic aromatic hydrocarbons (PAH) are toxic pollutants widely distributed in the environment. Organic amendment of PAH contaminated soil with compost and farmyard manure has been proven for efficient PAH bioremediation facilitated by native microorganisms, however, information on the identity of PAH degraders in organic-amended soil is still limited.
Here we show the PAH carbon turnover mass balance in microcosms with soil-compost mixtures or in farmyard fertilized soil using [^{13}C6]-pyrene. Complete pyrene degradation of 100 mg/kg of soil was observed in all supplemented microcosms within 3 to 5 months, and the residual ^{13}C was mainly found as carbon converted to microbial biomass. Long-term fertilization of soil with farmyard manure resulted in a similar pyrene removal efficiency, although with a much longer lag phase, higher mineralization and lower carbon incorporation into the biomass than amendment with compost. We aimed at providing molecular insight into the bacterial communities in soil amended with compost or farmyard manure and identiying the bacterial genera capable of degrading pyrene. We combined phospholipid fatty acid stable isotope probing (PLFA-SIP) to trace the carbon within the microbial population and the amount of biomass formed from pyrene degradation with a statistically evaluated bacterial genera abundance assessment based on total RNA and DNA extracted from the soil samples.
Complex pyrene degrading communities with low abundance of individual degraders were found instead of the expected single or few key players. In addition, pyrene mineralizing bacteria isolated or enriched from the soil compost or soil farmyard manure samples did not dominate pyrene degradation in the amended soils. The bacterial degrader communities of the soil-compost mixture and soil fertilized with farmyard manure differed considerably in composition although showing similar degradation kinetics. The data were also used to set up and validate an integrated degradation model. The results demonstrate that complex microbial degrader consortia rather than key players are responsible for PAH degradation in organic-amended soil. Organic amendments either as long-term manure fertilization or as one-time compost amendment play a key role in increasing the PAH-degrading potential of the soil microbial community.

Microbial community dynamics in the rhizosphere of a heavy-metal hyper accumlator

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Phytoextraction is a low-cost, green technology that uses heavy metal hyper-accumulating plants to remove heavy metals from soils. Interest in phytoextraction is intensifying as arable land becomes increasingly scarce.
Plant-growth-promoting rhizosphere bacteria (PGPR) can be used as an inoculum to improve phytoextraction. However, this strategy often focus on a single PGPR species whereas the rhizosphere contains a whole community of microbes that are part of a complex web interactions. This research aims to utilize whole soil microbial communities as a strategy for improving the phytoextraction of heavy metals by stimulating plant growth.
We report on a structural and functional investigation of microbial communities associated with a recently identified Australian native Cd-hyper accumulator. Bacterial and fungal communities were investigated using traditional culturing as well as community fingerprinting and 16S rRNA metagenomic profiling.
This research has identified twenty PGPR from the Cd-contaminated rhizosphere that exhibit a range of plant-growth-promoting attributes as well as heavy metal tolerance. Preliminary work correlating microbial plant-growth promoting abilities with microbial plant growth promotion in situ have identified phosphate solubilisation as a metabolic process of interest for whole-community functional analysis.
We demonstrate, using community fingerprinting, that bacterial and fungal communities operate independently of one another, responding to different selective pressures in the environment. We present evidence that heavy metal contamination, as opposed to the plant itself, is the predominant driver of rhizosphere community formation. Microorganisms that predominate in both rhizosphere communities and polluted soils are currently being identified via next-gen sequencing. Recently developed metagenomics software (PICRUSt) will be used to investigate the relationship between community structure and community function, and also identify candidate microorganisms of ecological importance.
Understanding factors that direct the formation of rhizosphere communities in heavy metal contaminated soils, and how they could be driven to promote plant growth, will be important for the development of phytoextraction. With the assistance of the correct microbial community, easily harvestable high-biomass crop species could be used to improve phytoextraction efficiency, even if the plants have no native hyper-accumulation ability.

Impact of mycelia-like dispersal networks on bacterial spatiotemporal dynamics linked to biodegradation at varying water potentials

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Bacterial population dispersal is a prerequisite for efficient biodegradation in soils because it increases the contact probability of bacteria and contaminants. However, in terrestrial environments bacterial surface motility is usually restricted due to a limited thermodynamic availability of water. In the porous soil matrix the osmotic (?o) and the matric potential (?m) constitute the main descriptors of the water potential and both are described to decisively affect bacterial motility behavior. Experimental studies and a spatially explicit microbial simulation model have proven that fungal mycelia facilitate bacterial dispersal in water unsaturated environments, which resulted in an enhanced contaminant degradation. Nevertheless, poor knowledge exists on the beneficial effects of mycelia at varying ?o and ?m. We therefore established experimental microcosms to investigate the effect of mycelia-like dispersal networks on the spatiotemporal dynamics of Pseudomonas putida KT2440-gfp at ??o and ??m between 0 and -1.5 MPa (i.e. water potentials representing completely saturated or plant permanent wilting point conditions) and determined their benefit for the biodegradation of benzoate as a model substrate. We found that a decrease of the osmotic potential slowed down bacterial dispersal and growth in the system. Consequently, biodegradation rates dropped by 50 % at ??o -0.5 MPa and by 90 % at ??o -1.5 MPa. In contrast, matric stress completely repressed bacterial movement already at ??m -0.25 MPa, which leads to a drop of the degradation rates by 40 %. Dispersal networks accelerate bacterial movement in all treatments and thus markedly improve the biodegradation performance by up to a factor of 4 and 1.8 for osmotic and matric stress treatments, respectively. We propose that dispersal networks may act as an important buffer mechanism for fluctuating water regimes in soils and hence increase the functional resistance of the microbial ecosystem.

Soil - sediment microbial community adaptation due to a long history of oil contamination

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Oil contamination due to anthropogenic activity can persist in the environment for many years with severe environmental impact. Natural oil seeps offer a unique opportunity to study the evolution and adaptation of ecosystems to long-term contamination and the biodegradation potential of indigenous microorganisms. Most oil seeps investigated so far are located on the seabed, thus there is little data available about microbial communities in other environments.
Here, we investigate a natural coastal oil seep located in Zakynthos Island, western Greece, called Keri Lake. In this unique ecosystem, flow of tar and petroleum products to the surface has been observed for more than 2000 years. We hypothesize that a well-adapted microbial community has been shaped under the long presence of oil hydrocarbons in soil. In order to study its structure and functional potential related to hydrocarbon biodegradation, replicate soil cores up to 7 meters were collected in October 2013 from highly- and non- contaminated areas in Keri Lake. The cores were sampled in situ in various depths spanning from ~0.1 to 6.5 meters.
The influence of hydrocarbons and sampling depth on microbial communities was assessed using direct sequencing of the different metagenomes. Our data revealed a significant influence of the contamination on community diversity. Both sites were dominated by Bacteria but a clear increase of archaeal sequences was observed in the oil contaminated samples. Whereas methanogenesis plays a strong role at the contaminated side, processes like sulfate reduction and denitrification are slightly inhibited.
To confirm these observations the obtained molecular data was further correlated to abiotic soil parameters and the chemistry of the oil.

Biochar for soil carbon sequestration and P fertilization: effects on microbes and fauna

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Thermal gasification converts biomass into a combustible gas in an oxygen-poor environment, the bi-product being biochar. The gas can be used to produce electricity and heating, as thermal gasification exploits 90-95% of biomass energy. The biochar can be used as soil amendment to increase pH and soil carbon content. Depending on the gasification conditions, nutrients like phosphate and potassium in the biochar are also plant available and will act as fertilizers.
The objective was to risk assess the potential effects of biochar amendment to agricultural soils on soil ecosystem services especially biodiversity and carbon sequestration to mitigate climate change. Specifically, we assessed effects on soil microorganisms and fauna (protists and earthworms). Biochar was added in amounts corresponding to the plant P demand (2-8 tons ha^{-1} $year^{-1}$); crops were alternating oil seed rape and winter wheat and biochar was added for 3 consecutive years to a Danish agricultural clayey to sandy soil with SOM content of ca. 3%. Earthworms and soil were sampled in October 2014 from field plots either left untreated (negative control), or amended with biochar; soil was sampled from bulk soil and the lining of earthworm burrows (the drilosphere).
Only one earthworm species increased in abundance in the biochar amended soil, while generally no effect of biochar was seen. Soil protists abundance increased in the drilosphere, however, in biochar amended soil the abundance decreased in the drilosphere while basal soil respiration was always higher in the drilosphere. Culturable bacteria and enzymatic activities of sulfatase, phosphatase and carbohydrate degrading enzymes did not show significant differences. Metagenomics of bacterial and protest communities (16S and 18S rDNA, respectively) will describe the community biodiversity.
Generally, the priming effect of the drilosphere was larger than any effect of biochar amendment on the soil microorganisms and earthworms. These results show that in the tested agricultural soil the addition of biochar according to the plant P demand had limited effect on soil microorganisms and fauna, and could be a sustainable fertilizer mitigating climate change by increasing soil carbon content.

The microbial origin of humic substances: In honory of Konrad Haider and James P Martin

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Konrad Haider and James Martin have conducted researches from 1969 to 1988 studying the formation, composition and functions of soil organic matter and the fate and interaction of organic pollutants with humic molecules. Here we shall discuss their hypothesis on the fungal origin of humic molecules. They observed that *Epicoccum nigrun*, *Stachbotrys atra* and *Stachbotrys chartarum* were capable of synthesizing and then oxidizing phenols in the presence of different amino acids compounds with formation of humic-like substances. Haider and Martin suggested that the secondary metabolism was responsible for the formation of phenolic polymers. Almost 50 years later Schnitzer and Monreal (2011) proposed the synthesis of humic molecules from low molecular weight organic compounds through the synthesis of polyketides by the secondary metabolism. Polyketides comprise a large variety of natural products, some of them with biological activity (e.g. antibiotics), which are synthesized from acyl CoA precursors by reactions including that catalyzed by polyetide synthase. These compounds can provide antihelmintic, insecticidal, antibiotic and antienzymatic properties as well as they can be pigment or provide regulatory functions.
Nowadays there is a scientific dispute on the composition and formation of humic molecules with critical comments on the soil extraction approach and separation in humic and fulvic acids. The dark color is the basic characteristics of humic substances. Melanin, a high-molecular weight and dark coloured pigment produced as a secondary metabolite by fungi, has been considered a possible component of humic substances also considering that dead melanic fungi are more resistant to degradation in soil than dead non-pigmented fungi. Future research should verify the importance of secondary metabolites, including melanin, in the formation of humic substances and soil organic matter in general.

Microbial Colonisation and Resource Partitioning in Agricultural Soils

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Microbial assimilation of soil organic carbon is an important process in global carbon cycling, since it determines the magnitude of microbial biomass in soils and controls processes leading to soil carbon stabilisation. Mechanisms and controls in microbial carbon assimilation play also a fundamental role in regulating land-atmosphere interactions. Nevertheless, microbial biogeochemistry is still one of the greatest uncertainties in Earth system models. Therefore, capturing key aspects of microbial mechanisms (like e.g. the differences in bacterial and fungal physiology) is an urgent requirement to improve these models. Whereas direct linkage of genomes to global phenomena is still a great challenge, many connections at intermediate scales are viable with integrated application of powerful analytical and modelling techniques. This integration could enhance for example management strategies for capturing and sequestering atmospheric CO_2 and/or evaluate thermal adaptation of decomposer communities in warming soil. Recent studies of our group disentangle the herbivore and detritivore pathways of microbial resource use at soil biogeochemical interfaces, identify the key players contributing to these two different pathways, and determine to what extent microbial substrate use is affected by environmental controls. To follow the kinetics of litter and root decomposition and to quantify the contribution of key players, we used isotopic approaches like PLFA-SIP and ergosterol-SIP. Bacteria and sugar consuming fungi initiated litter decomposition in a microcosm experiment during the first two weeks, whereas higher fungi started to grow after the depletion of low molecular weight substrates. In second series of experiments we investigated the mechanisms that could contribute to the development of spatial heterogeneity in soil microbial communities in grassland soils: (1) colonization of new surfaces (minerals, organo-mineral complexes and litter) and (2) resource partitioning between bacteria and fungi in the detritusphere and the rhizosphere. These experiments provide novel insight into the physical and resource niches occupied by soil microorganisms.

Ectomycorrhizal fungi drive humus decomposition in boreal forest

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In boreal forest soils, mycorrhizal fungi play a prominent role among soil microbiota. The biotrophic mycorrhizal fungi have previously been assumed to have lost most of the decomposer capacity characteristic of their saprotrophic basidiomycete ancestors. Here, field observations are presented to support that some ectomycorrhizal fungi have retained capacity to degrade recalcitrant organic matter. Decomposition is proposed to gain mycorrhizal fungi increased access to limiting nitrogen rather than metabolic carbon analogous to a “priming effect” facilitated by easily utilised host sugars.
High throughput sequencing of fungal ITS2 markers from old-growth forests showed a negative correlation between soil carbon stocks and DNA of certain ectomycorrhizal fungi, primarily of *Cortinarius* – a species rich and often dominant genus in boreal forests. Sequencing of a *Cortinarius* genome revealed a relatively high number of genes coding for Mn-peroxidases – potent oxidative enzymes that are by saprotrophic basidiomycetes to degrade lignin but have largely been lost during evolution of other ectomycorrhizal lineages. Mn-peroxidase activity was found to correlate negatively with soil carbon stocks, supporting a key role in the regulation of carbon sequestration at the ecosystem level. Exclusion of ectomycorrhizal fungi from field plots by cutting root sugar supply resulted in a loss of manganese peroxidase activity in the humus layer. In contrast, hydrolytic enzymes yielding metabolically useful products were little affected by root severing and were spatially restricted to surface litter dominated by free-living saprotrophs.
These results indicate that a limited group of ectomycorrhizal fungi play a key role as regulators of organic matter persistence in boreal forest soils, due to their capacity to combine a biotrophic life strategy with potent oxidative enzymes in a “symbiotic priming effect”. Low abundance of these fungi, e.g. due to stressful conditions, promotes organic matter accumulation with a feed-back on soil fertility and ecosystem production that lead to ecosystem retrogression. Many ectomycorrhizal fungi – particularly members of the genus Cortinarius – are sensitive to environmental disturbances, such as nitrogen deposition or intense forestry. Recognition of their central role as decomposers enable better predictions of responses of soil fertility and carbon sequestration to forest management, pollution and climate change.

What do they eat? Exploring the substrate-specificity of microbial communities in the decomposition of C sources in a forest soil

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Forest soils are among the most important terrestrial carbon sinks. In these ecosystems, carbon is mainly found in the dead plant biomass due to yearly fallen litter as cellulose, hemicellulose and pectins, but also in other biopolymers such chitin and peptidoglycans from fungal and bacterial origin respectively. Microbial communities inhabiting in soil decompose these polymers into sugars that are used as energy source, being the main drivers of carbon efflux in the ecosystem and playing critical roles in the reconversion and recycling of nutrients. For these reasons, the decomposition process in forest is a central point of the ecosystem functioning and concerns a key step in the global carbon cycle. In this way, the study of the structure and composition of these communities may help to indicate which taxonomical groups are involved in the decomposition processes and how specific they are. In this work, we used a SIP approach with six different ^{13}C-substrates (glucose, cellulose, hemicellulose, plant biomass, bacterial biomass and fungal biomass) in microcosms containing forest soil. Microcosms were incubated and collected at 0, 7, 14, and 21 days, and used for ^{13}C analyses in CO_2, PLFA and separation of ^{13}C and ^{12}C-DNA. Both bacterial and fungal communities were analyzed by amplicon sequencing (Illumina) of 16S rRNA gene and ITS region of ^{13}C and ^{12}C-DNAs. Results showed that the decomposer communities are substrate-specific in their composition, offering evidence about the substantial contribution of bacteria together with fungi in the decomposition processes in forest soil.

Similar and contrasting patterns of natural fungal community assembly during initial decay of coniferous and broadleaf tree logs: an experimental common garden approach

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Fungal community composition can influence the rate of decomposition. Since fungal communities are dynamic, community assembly processes do not only influence community structure, but also affect decomposition processes. We hypothesized that fungal community assembly processes in decaying logs are influenced by 1) the presence of established fungi, causing priority effects, by 2) random effects such as random dispersal of spores having equal chances to colonize the community or by 3) combative abilities among fungi, resulting in high relative abundance of the best competitor. To test these hypotheses we investigated fungal community assembly processes using 454-pyrosequencing during decay of freshly cut logs of the coniferous *Larix kaempferi* (Lambert) Carri?re and the broad-leaf tree *Quercus rubra* (L.) in a common garden approach to minimize the effects of abiotic environmental variation. Decomposer fungal community composition shifted drastically over time and varied greatly between *Larix* and *Quercus* logs, with both similar and contrasting patterns of fungal community assembly. Endophytic fungal communities could only be well analyzed for *Larix* and were found to be highly diverse and variable among trees, but composition of fungi that were present after one year of wood decay did not depend on endophytic community composition. After one year, we found a few dominant fungal species and similar composition of fungal communities among logs for both tree species. This indicates that priority effects of primary species on the community development on logs were small under these natural conditions. Instead, random effects in combination with the presence of combative fungal species seem to explain the assembly processes. During the second year of decomposition, an increase in variation in fungal community composition among *Larix* logs was observed, without apparent priority effects. In contrast, in many of the *Quercus* logs a few early colonizers were still dominating fungal community composition, and some species always co-occurred. We conclude that during prolonged decomposition, an increase in the patchiness of wood quality influenced fungal interactions in *Larix* and together with the random arrival of spores, resulted in unpredictable community assembly patterns. In *Quercus* logs, however, community assembly patterns were more predictable and indicated the occurrence of priority effects. These results will be discussed in relation to carbon cycling.

Redistribution of soil water by saprotrophic fungi enhances carbon mineralization

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Summer droughts are common in temperate forests and especially the upper soil horizons experience soil drought. Drought events can be accompanied by negative effects for forest ecosystems but many plants can reduce drought stress by hydraulic redistribution (HR). Similar processes were recently described for ectomycorrhizal networks but no information is available for mycelia networks of saprotrophic fungi. They strongly contribute to belowground nutrient cycling, C and N mineralization and fungal communities were shown to be less sensitive and better adapted to soil desiccation than bacteria communities.
We hypothesize that redistributed water by saprotrophic fungi triggers mineralization of organic matter in soils under drought conditions.
The impact of HR by saprotrophic fungi on mineralization was determined using mesocosms comprising two chambers, separated by a 2 mm air gap to prevent bulk flow of water. After inoculation with fungal cultures and a growth phase, both chambers were desiccated. Subsequently, only chamber I was rewetted while chamber II was treated with ^{13}C labelled plant material. CO2 samples were collected over 7 days after rewetting and analyzed for stable isotope ratio. In addition, enzymatic activity of chitinases and cellobiohydrolases in chamber II was determined after 7 days using the soil zymographie method. HR was prevented in the controls by trenching the hyphal bridges between the two chambers.
HR led to a strong increase in volumetric water content in chamber II after rewetting of chamber I. The increase in soil moisture by HR strongly enhanced carbon mineralization and enzymatic activity in chamber II. Our results demonstrate that mycelia networks of saprotrophic fungi contribute to redistribution of water from wet to dry soil. HR can partly compensate water deficiency if water is available in other zones of the mycelia network and is likely a mechanism for higher drought resistance of soil fungi compared to bacteria.

Elucidating microbial drivers of decomposition and the carbon cycle through a co-occurrence framework

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The fundamental problem with determining links between microbes, carbon (C) cycling, and climate system feedbacks is that soil microorganisms are functionally and phylogenetically diverse due to the variety of soil C-substrates. Traditionally, microbial C-cycling has been summarized by extracellular enzymes, which are proximal indicators of decomposition. Next-generation sequencing technologies are also now providing massive amounts of data characterizing what microorganisms are present (marker gene sequencing) and what traits they harbor (metagenomes). Integrating these datasets, we have asked the questions: Which microorganisms coexist and potentially interact to drive C-cycles, what C-cycling traits are involved, and are these related to our biogeochemical indicators of decomposition? Using a Bayesian co-occurrence framework, we combined datasets to answer these questions in two different ecosystems: soil aggregates isolated from agricultural systems and decomposing logs in forests. When analyzing co-occurrence networks, we identified fungi that may ultimately be responsible for producing the extracellular enzymes measured commonly in laboratory assays. Several fungal taxa including members of *Claviceps*, Orbiliaceae, and Phaeosphaeriaceae increased linearly in abundance with sequences associated with enzyme families including cellulases and cellobiohydrolase. Independent analyses of genomes representing these fungal taxa confirmed the presence of cellobiohydrolase genes (*cbhA*), confirming their potential role in soil C-cycling. Among decomposing logs, co-occurrence relationships between fungal taxa and extracellular enzymes were most common among later stages of decomposition, though this depended on tree species. Several fungal taxa co-occurring with greater cellobiohydrolase activity belong to the saprobic order, Helotiales. Representative genomes from this group were also identified as containing cellobiohydrolase genes (*cbhB*) as well. Overall, these results suggest that co-occurrence analyses can identify putative relationships between microbes and enzymatic potential while identifying potential drivers of decomposition and C-cycling.

Spectroscopy and transcriptomics provide novel insights into soil organic matter decomposition mechanisms in ectomycorrhizal fungi

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Decomposition of soil organic matter (SOM) is thought to involve saprotrophic fungi and not symbiotic fungi, which include ectomycorrhizal fungi; the latter are instead thought to bring plant carbon into the soil. This view is supported by the loss in ectomycorrhizal fungi of many genes encoding lignocellulose-degrading enzymes in their saprotrophic ancestors. However, we demonstrate here that ectomycorrhizal fungi can decompose SOM in the presence of glucose. Using spectroscopy, we show that five species of ectomycorrhizal fungi, representing at least four clades that have independently evolved symbiosis, have substantial capacity to decompose SOM extract using oxidative mechanisms. RNA-Seq analyses revealed that the genome-wide set of transcripts expressed during litter decomposition has diverged over evolutionary time. Each species expressed a different set of enzymes that are associated with oxidative lignocellulose degradation by saprotrophic fungi. The decomposition "toolbox" has diverged through differences in the regulation of orthologous genes, the formation of new genes by gene duplications, and the recruitment of genes from diverse but functionally similar enzyme families. A comparison of closely related species within the Boletales clade showed that ectomycorrhizal fungi oxidized components in the SOM extract as efficiently as brown-rot wood-decayers. The ectomycorrhizal species within this clade exhibited more similar decomposing mechanisms than expected from the species phylogeny in concordance with adaptive evolution occurring as a result of similar selection pressures. We propose that the ancestral decay mechanisms used primarily to obtain carbon have been adapted in symbiosis to scavenge nutrients rather than releasing carbon. However, when supported by energy from the host plant, the oxidative decomposing activity of ectomycorrhizal fungi may significantly affect the chemistry of organic molecules in SOM, which could influence fundamental soil functions including the turnover and stabilization of organic matter.

Microbial regulation of carbon cycle: evidence from gene-enzyme relationship

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The response of soil process and carbon (C) cycling to environmental change depends on the responses on complex soil microbial communities; however the microbial activity is only implicitly represented in most Earth Ecosystem Models (ESMs). It has been postulated that developing improved models of microbial processes will generate more accurate projections of soil C feedback on climate change and reduce source of uncertainty in current ESMs. The lack direct and quantitative evidence of microbial regulation of ecosystem processes including C degradation hinders our ability to develop a framework to directly link ESMs to genetic composition of microbial communities. Using 51 samples across all three geographical regions of Australia, which varied in climatic, abiotic and biotic properties, we examined the linkage between microbial community and activity of enzymes directly linked to C degradation. Results show strong correlation between microbial functional genes and activity of four enzyme activities related to C cycle (R2 = 0.89-0.99). Structural Equation Modelling (SEM) analyses explained most of the variation in the activity of enzymes involved in C degradation which is mainly predicted by the functional structure of soil microbial community (R2> 0.90 in all cases). Finally, multi-model analysis showed that incorporation of microbial community function and composition accounted for 88-97% of variation and provided best fitting models to predict the activities of enzymes. We provide empirical evidence of the microbial regulation of soil processes linked to C degradation in terrestrial ecosystems. The extremely strong correlation between C degrading enzymatic activities with functional gene further suggest that soil microbes are good proxy to soil functionality and can be predicted based on the available information on the microbial community composition. Our study provides a strong framework for improved predictions which could be achieved by adopting gene-centric approach incorporating the abundance of functional genes into process models.

Mycorrhizas: Pipeline or Director of Belowground C Fluxes?

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Understanding the fate of carbon (C) allocated to the soil is important to influence C sequestration in ecosystems. Roots direct resources in form of carbohydrates to nutrient patches, and both their biomass and exudates fuel carbon compounds into the soil spaces they are exploring. What role do their root symbionts mycorrhizal fungi play? Do arbuscular mycorrhizal fungi (AMF) constitute a nutrient patch for the plant on a root cellular level? Can AMF be viewed as the extended phenotype of the root, or do they give additional attributes to the carbon distribution and stability in soil?
We found that AMF, or roots through AMF, directed 5-fold more ^{13}C to OM patches, compared to plain soil in a field experiment. While in a pot experiment AM fungal colonization did not alter the total amount of ^{14}C belowground allocation of the model grass species Brachypodium distachyum, there was a strong preferential C allocation to the AM colonized root part of split-root systems, receiving double the amount of C than the non-colonized root system side. This preferential carbon allocation to mycorrhized roots stopped abruptly when P limitation of the plant was lifted, demonstrating the differential, targeted C belowground allocation during nutrient foraging of the plant.
While these results point to a plant directed C flux to nutrient patches via AMF on a centimeter scale, we also found evidence that on micrometer scale there may be processes in C allocation which are special to AM fungi: We found that the 13C coming from a donor plant to an AM fungus was substantially allocated to the intraradical mycelium in a neighboring C-starved root. This could be interpreted as using a C-starved root as a storage space safe from predators. We also found hyphae colonize remote pore spaces of black carbon particles which are inaccessible for plant roots, and likely unattractive to decomposers. Such carbon compounds may become ‘protected’ against decomposers, and may be more recalcitrant simply due to its location in soil space. Thus, we want to discuss the importance of the spatial location of sequestered C in soil aggregate space for its long-term stability.

The Hidden World Within Plants: Ecological Considerations and Functioning of Microbial Endophytes

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All plants are internally inhabited by diverse microbial communities comprising bacterial, archaeal, fungal, and protistic taxa. These microorganisms showing endophytic lifestyles play a crucial role in plant development, growth, fitness and diversification. They actively participate in plant growth promotion and protection against biotic and abiotic stress, e.g. by triggering the host induced systemic resistance response, production of antibiotic and other secondary metabolites, iron homeostasis, osmotic and stomatal regulation, synthesis of phytohormones, 1-aminocyclopropane-1-carboxylate deaminase and volatile organic compounds, assimilation of nutrients and fixation atmospheric nitrogen. They might also drastically affect host plant morphology and physiology, including gender selection. The increasing awareness and information on endophytes provides insight into the complexity of the plant microbiome. The nature of these interactions ranges from mutualism to pathogenicity. Here, we revisit the literature to access the historical evidences reporting endophytes and demonstrate that it is not trivial to clearly distinguish a non-pathogenic endophyte from a pathogen. Properties such as pathogenicity or mutualism may depend on many factors, including the genotypes of plants and microbes, environmental conditions and the dynamic network of interactions within the plant biome. By considering the latest insights into evolution, plant ecosystem functioning and multipartite interactions, we address the concept of endophytism. We then question the currently applied definition of endophytes and claim that the term "endophyte" should refer to only habitat and not function. By implementing new technologies and multi-disciplinary approaches, our understanding on endophyte biology and ecology will consistently evolve further, leading to a better knowledge of the plant holobiome.

The effect of soil fauna in decomposition activity of soil microflora

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Meta-analysis of various enclosures these experiments indicated that soil fauna enhanced removal from soil surface but did not significantly affect overall C mineralization. This is consistent with meta-analysis showing that rate of leaf litter decomposition is significantly faster than decomposition of macrofauna feces produced from the same litter. In soil affected by earthworms the coating of organic matter by clay is often assumed to be important driver of SOM stabilization. This effect may depend on duration of fauna activity in soil. In long term laboratory experiment C loss from the laboratory microcosms where liter was either mechanically grounded and mixed in to the soil or mixed into the soil by earthworms (Lumbricus subellus). Earthworm treatment show significantly the highest respiration during first c. 20 weeks of experiment in intermediate stages of experiment there were no significant differences between treatments. In 80-126 week of experiment mechanically mixed soil show significantly higher respiration than earthworms. These results are consistent in both litter and substrate combinations. C budget in both layers after 126 weeks shoe the highest C stock in surface applied liter but most litter was on soil surface. The experiment show that incorporation of organic matter in to soil by earthworms result in higher C sequestration than mechanical mixing that may imitate tillage or other form of cultivation. Besides coating also chemical changes of litter can contribute to slow down of litter decomposition. To understand chemical changes in excrements that may because they decompose more slowly than leaf litter, we fed Bibio larvae the litter of tree species differing in litter quality (alder willow and oak) and then measured respiration induced by litter and excrements. We also measured respiration induced by the same litter artificially modified to mimic faunal effects; the litter was modified by grinding, grinding with alkalinization, grinding with coating by kaolinite, and combination of both alkalinization and coating. Our findings indicate that the decreased decomposition rate of excrements might result from the removal of easily available polysaccharides, the increase in aliphatic components, an increase in the resistant components of lignin and the binding of nitrogen into complexes with aromatic components. Several of these mechanisms are supported or determined by liter alkalinization during gut passage.

Recruitment of a beneficial rhizosphere community by the common dandelion (*Taraxacum officinale*) from different soil types

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Relationships between plants and their microbes are specialized such that plant species-specific rhizosphere communities tend to be different from even the bulk soils immediately surrounding the plant. In order to investigate the dynamics of the plant-microbe relationship across a wide range of ecosystems, microbial recruitment by the ubiquitous invasive weed, the common dandelion (*Taraxacum officinale*) was studied. The objective of the study was to determine the ability of surface-sterilized dandelions to recruit their associated rhizosphere community out of different soils. After collection from a central location, dandelion roots were surface sterilized and transplanted into 30 different soils sampled from mountains, clay-rich piedmont, interior sandhills, and sand-rich coastal regions. Rhizosphere communities were assessed after 1 and 4 weeks of growth and compared to bulk soil and unmanipulated dandelions. Bacterial and microeukaryotic assemblages were determined by amplifying portions of the 16S and 18S rRNA genes, respectively, and sequencing amplicons on the Illumina platform. One week after sterilization and transplantation, microbial communities in the rhizosphere were not distinct from those in source soil ($p = 0.94$) and were significantly different from non-transplanted dandelion controls ($p = 0.001$, after Bonferroni correction $p = 0.008$). In these samples, bacterial indicator taxa were dominated by *Agrobacterium* spp., a lineage known to induce plant tumors. Four weeks after manipulation, however, the relationship between rhizospheres and bulk soil, while not significantly different, were no longer as strongly associated ($p = 0.10$). Interestingly, microbial communities of transplanted dandelions began to more closely resemble those of unmanipulated dandelions and were no longer significantly different ($p = 0.02$). Shifts in indicator taxa of the 4-week samples were evidenced by an increase in the community contribution of *Bradyrhizobium* spp. and *Methylibium* spp. - two genera that include nitrogen-fixing bacteria. Results to date suggest dandelions are capable of recruiting beneficial microorganisms out of diverse soil conditions, possibly contributing to the species' invasive success. This experiment is currently continuing, in order to monitor shifts in microbial community diversity and function across the life stages of the plant to gain further insight into microbial recruitment in the dandelion rhizosphere.

The sapro-rhizosphere concept: Bacteria as secondary consumers of plant-derived carbon

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In soil food webs microorganisms are traditionally considered as a distinct level of the food chain, namely, as primary consumers of root-derived organic compounds. Trophic interactions among primary consumers could however induce shifts in food web energy flows and belowground nutrient cycling. Given the increasing evidence for primary consumption of root exudates by saprotrophic fungi, we propose that fungus-derived carbon may be an important resource for rhizosphere bacteria. Here, we present the "sapro-rhizosphere concept", stating that many rhizosphere bacteria are actually able to feed on root-derived compounds as secondary, rather than primary consumers.
To test this concept, two common saprotrophic, rhizosphere-inhabiting fungi, *Trichoderma harzianum* and *Mucor hiemalis*, were confronted in microcosm systems with bacterial communities extracted from the rhizospheres of two plant species, *Carex arenaria* and *Festuca rubra*. This showed a widespread ability of rhizosphere bacteria to attach to and actively feed on living hyphae of saprotrophic fungi. The identity of the fungi had a strong effect on the composition of these potential mycophagous bacteria, whereas the effect of plant species identity was small. Based on our results, we suggest that food web models should take the possibility of bacterial secondary consumption into account as this has important consequences for and carbon fluxes, with more carbon dioxide released by microbes and less microbial carbon available for the soil animal food web. Additionally, we found an unusually high amount of potentially plant pathogenic bacteria to be associated with fungal hyphae. This might suggest a supporting fungal role in bacterial plant diseases.

Forest microbiome - diversity, functioning and dynamics

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Forest ecosystems are of global importance as recent C sinks and pools. However, their behaviour in the future is largely unpredictable due to complexity of their functioning involving trees as dominant primary producers, microorganisms as major decomposers and other guilds of organisms. Despite intensive research, the functioning or microorganisms in the ecosystem is not fully appreciated. This is mainly due to simplistic approaches that often target only selected compartments of the ecosystem such as the soil or tree roots. In reality, the complexity of the ecosystem is much higher and the niches are often unique and have specific properties. The microbiomes of tree or plant leaves and living tissues, roots, deadwood, leaf and root litter, mycelial mats in soils, tree-associated invertebrates, rhizosphere and bulk soil as well as weathered mineral horizons are each highly specific in terms of gross chemistry, nutrient availability, and dynamics. Recent papers make it possible to provide a first view of the composition of microbial communities inhabiting these individual niches, allow us to formulate the potential major processes driving their existence and functioning. It is also possible to outline the model of their dynamics on the scales ranging from day/night and annual cycles, to decomposition of dead organic matter of various recalcitrance and ecosystem development itself. The microbial communities inhabiting soil, plant litter, rhizosphere and roots of forest plants, deadwood or aboveground plant tissues differ tremendously in the drivers of their assembly and consequently in diversity and composition, functioning and dynamics in time which is specific for each habitat. Due to this, to achieve the understanding of the microbial contribution to the functioning of the ecosystem, the analysis has to be done on the „ecosystem microbiome“ level, which, in parallel to the „human microbiome“ should point at the complexity and diversity of the system.

Giants of the soil microbial world: foraging cord-forming fungi

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The majority of soil fungi spend the majority of their lives as dormant spores or feeding mycelia. Some saprotrophic wood decay basidiomycetes spread spatially and temporally as extensive, long-lived mycelia systems, ramifying through forest floor soil and litter. These mycelia are extremely effective dispersal structures, and operate foraging strategies enabling capture of resources patchily and sparsely distributed in space and time. Their main resource capture strategies are: (1) 'sit and wait', whereby a large mycelial network waits for resources to land on it; (2) 'seek and find' where the mycelium grows out of a resource in active search for new resources; and (3) most commonly both (1) and (2) simultaneously. Mycelial networks respond dramatically to encounter with new resources, and are remodelled continuously. Further, essential carbon and mineral nutrient sources are translocated around these networks to places where they are needed. Their success lies in this ability to forage successfully, to move nutrients around, to remodel, and in their network architecture, both which vary between species and environmental conditions. This talk will reveal the dramatic ways in which saprotrophic cord-forming basidiomycetes respond to new resources and to interactions with the abiotic and biotic environment, by remodeling mycelial architecture and taking up and translocating nutrients.

A decade of irrigation transforms the soil microbiome of a semi-arid pine forest

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Impacts of climate change on the soil microbiome potentially alter biogeochemical cycles of terrestrial ecosystems. In semi-arid environments, water availability is a major constraint on biogeochemical cycles due to the combination of high summer temperatures and low rainfall. Here, we used a high-throughput sequencing approach to explore how ten years of irrigation of a water-limited pine forest in the central European Alps altered the soil microbiome and associated ecosystem functioning. A decade of irrigation stimulated tree growth and photosynthetic activity, resulting in higher crown cover, larger yearly increments of tree biomass, and greater root biomass. Increased primary production in the irrigated forest stands promoted rhizosphere priming, induced by greater amounts of plant-derived inputs and shifts in the microbiome from largely oligotrophic to more copiotrophic lifestyles. According to the current framework of soil heterotrophs, bacterial and fungal groups benefiting from increased resource availabilities (litter, rhizodeposits) thrived under irrigation, leading to enhanced soil organic matter mineralization and carbon loss from the organic horizon. The loss of soil carbon due to increased microbial activity in the irrigated stands was contrasted by higher primary production and enhanced carbon sequestration to the extent that it fully compensated for the increased soil organic matter decomposition. This unique long-term study provides new insights into the impact of precipitation changes on the soil microbiome and associated ecosystem functioning in a drought-prone pine forest ecosystem. Such insights improve our understanding of the soil carbon turnover and the associated implications for long-term soil carbon stocks in a changing climate.

Genotypic Diversity Matters: Examining the diversity-ecosystem function relationship with ectomycorrhizal fungi

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The relationship between biodiversity and ecosystem function is a hotly debated topic in ecology and yet little is known about the nature of the relationship with respect to forest soil microorganisms. Furthermore, even less is known about the relative importance of within-species versus between-species diversity. Ectomycorrhizal (ECM) fungi are important members of forest soils having key roles in biogeochemical cycles and other important ecosystem processes, and can vary in their functional traits suggesting their potential for diversity effects on forest ecosystem functioning. To bridge this gap in knowledge, the following hypotheses were tested: 1) richness and identity of ECM fungal genotypes (intraspecific), relative to species (interspecific), regulate plant and fungal productivity, and have quantifiable effects on ecosystem processes (soil CO^2 flux and nutrient dynamics), and 2) the effects of genotypic ECM diversity on productivity are greatest when the chemical composition of nutrient resources in soil are complex, due to niche complementarity. To test for ecosystem responses, microcosms containing pine seedlings colonized by a diversity gradient, from monocultures to mixtures of ECM genotypes or species, were utilized. Significant genotypic identity effects on plant and fungal productivity, and nutrient loss in leachate were found. There was a positive genotypic richness effect with root biomass, root length and ECM root-tips per root length, and nutrient loss deceased with increasing genotypic richness. Genotype mixtures outperformed the monocultures in only half of the cases, suggesting that complementarity and selection affects are both in operation. In contrast, species monocultures mostly outperformed the mixtures. Overall, genotypic effects were as significant as species effects. Contrary to expectation, genotypic effects were less significant under complex versus simple soil nutrient resources. Stable isotopes labelling studies in microcosm and fungal culture experiments were utilised to further elucidate genotypic identity effects. Genotypes were found to vary in growth rate and nitrogen source uptake and utilization. Results highlight the importance of ectomycorrhizal fungi and intraspecific genotypic level of diversity in the regulation of forest ecosystem functioning.

Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems

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Many soils throughout the world are deficient in free, plant available phosphorus (P). However microbes are known to be effective in mineralizing organic and solubilizing precipitated forms of inorganic P. In this study we investigated the impact of soil P supply on microbial community structures and P cycle associated genes. We analyzed samples from two contrasting beech forest soils containing high (BBR) or low (LUE) amounts of total P. We assume that in a P-depleted soil the recycling of organic bound P is dominant whereas in a P-rich soil the solubilization of inorganic P prevails.
Direct metagenomics sequencing was carried out using the Roche 454 technology. Data analysis was performed implying MEGAN. Microbial biomass P as well as microbial carbon and nitrogen contents were additionally determined.
Taxonomic annotation of datasets revealed a domination of Proteobacteria (44.9% of assigned sequences), Actinobacteria (21.3%) and Acidobacteria (20.6%) in both soils. A surprisingly high abundance of Rhizobiales was detected in BBR (33.2%) compared to LUE (16.0%) ($P<0.0003$), which in turn was dominated by Actinomycetales (26.8%) ($P<0.113$) and Acidobacteriales (17.2%) ($P<0.0005$).
Most abundant P cycle associated genes referred to microbial phosphate transporters, including the highly efficient phosphate-specific transporter (*Pst*) and the phosphate-inorganic transporter (*Pit*). Both systems showed a higher abundance in the P-depleted soil. Genes involved in P starvation response regulation (*PhoB, PhoR*) were detected more frequently compared to enzymes performing the mineralization of organic P (e.g. acid and alkaline phosphatases). This demonstrates the importance of efficient microbial P uptake systems together with effective gene regulation under P limitation, allowing microbes to succeed in the struggle for phosphorus with plants. Regarding inorganic P solubilization, a significantly higher microbial potential was detected in the P-rich soil. This trait especially referred to Candidatus Solibacter usiatus, likewise one of the dominating species in the datasets. Moreover, predicted genes were primarily harbored by Rhizobiales (predominantly in BBR), Actinomycetales and Acidobacteriales (mainly in LUE). Direct targeting of the functional microbial community uncovered the hitherto unseen contribution of certain taxa to soil P cycling. Our results further corroborate the hypothesis that solubilization of inorganic P plays a major role in P-rich soils.

Microbial Community Response to Timber Harvest

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Forest harvest has the potential to alter the composition and activity of the soil microbial community. The effect of clear-cutting was studies at nine managed, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) dominated forests in the Pacific Northwest of the United States. The composition of bacterial and fungal microbial communities, pre- and post-harvest, were assessed by amplicon sequencing, and microbial activities were assessed by laboratory incubations and a suite of extracellular enzyme potentials. Microbial communities and activities varied across the nine sites and were related to soil and climatic factors. Both microbial community structure and activities were affected by harvesting, with structure being more resilient than activity.

Tree diversity and sampling effects on soil fungi, protists and meiofauna as revealed by multiplex ITS metabarcoding

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Plant species richness and the presence of certain influential species (sampling effect) drive the stability and functionality of ecosystems as well as primary production and biomass of consumers. Largely due to methodological constraints, very little is known about these floristic effects on richness and community composition of soil biota in natural habitats.
To accomplish this, our research consortium developed a DNA metabarcoding approach to identify the major eukaryote groups directly from soil with roughly species-level resolution. This method takes advantage of multiple mixed taxon-specific primers targeting the ITS2 subregion of ribosomal DNA and covering roughly 80% of eukaryote taxa. Using this method, we examined the effects of tree diversity and individual tree species on soil microbial biomass and taxonomic richness of soil biota in two experimental study systems in Finland and Estonia and accounted for edaphic variables and spatial autocorrelation.
Our analyses revealed that the effects of tree diversity and individual species on soil biota are largely context-dependent. Multiple regression and structural equation modelling suggested that biomass, soil pH, nutrients and tree species directly affect richness of different taxonomic groups. The community composition of most soil organisms was strongly correlated due to similar response to environmental predictors rather than causal relationships. On a local scale, soil resources and tree species have stronger effect on diversity of soil biota than tree species richness per se.
The methodological advantages of the ITS region-based multiplex metabarcoding design include 1) species-level resolution; 2) possibility to select and deselect eukaryote groups to be covered by optimizing the primer mix. The major disadvantages include 1) the paucity of reference ITS sequence data for several eukaryote groups and overly long ITS sequences in a few animal taxa. A parallel study using functional metagenomics approach revealed that metagenomics is not a viable alternative for biodiversity research and it has multiple unaccounted biases as well.

Microbial community shifts in structure and potential function across a boreal forest fire chronosequence

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Forest fire is a common natural disturbance in forested ecosystems and impacts heavily on forest soil microbial communities. Fungi are the predominant decomposers in forest soil and bacteria are the most abundant group of soil microbes. Both perform essential ecological functions in forested ecosystems via nutrient cycling and decomposition of organic matter. The response of soil microbial communities to forest fire is poorly documented. Here, we investigated the fungal and bacterial community structure and function across a 152 year boreal forest fire chronosequence (2, 42, 60 and 152 years post-fire) using high-throughput sequencing coupled with functional gene array (Geochips).

Our results demonstrate that the boreal forest soil fungal community was most diverse two years after the fire disturbance. The diversity of the fungal community subsequently decreased over time. The differences in the fungal community structure were explained by changes the abundance of Basidiomycetes and Ascomycetes. Ectomycorrhizal (ECM) fungi contributed to the increase of basidiomycete abundance over time with the OTUs representing the genera *Cortinarius* and *Piloderma* dominating. The site with the highest fungal biological diversity had also the most diverse fungal genes. The genes involved in the organic matter degradation were as common in the mature forest, where ECM fungi were the most abundant as the youngest site where saprotrophic fungi had relatively higher abundance.

In contrast, the bacterial diversity did not show difference between the recently burned and older burned forest. Proteobacteria (39%) and Actinobacteria (34%) were the most abundant groups across all sampling sites followed by Acidobacteria (19%), Bacteroidetes (2%) and a small portion of Planctomycetes, Armatimonadetes, TM7, Verrucomicrobia, Gemmatimonadetes and Firmicutes. Interestingly, the abundance of each bacteria group remained relatively stable over time.

Hierarchical cluster analysis (HCA) by using gene signal intensity revealed that the sites with different fire history formed separate clusters for both fungi and bacteria suggesting potential differences in the potential to maintain essential biogeochemical processes in soil. This study provides insight into the impact of fire disturbance on soil microbial community dynamics

Unravelling the ecological function of abundant but uncultivated Thaumarchaeota in acidic forest soils

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Thaumarchaeota are very abundant microorganisms and are found in a wide range of environments, including acidic forest soils. The most studied Thaumarchaeota are placed within Groups 1.1a and 1.1b and they play an important role in nitrogen cycling by performing ammonia oxidation. The function of other thaumarchaeotal phylogenetic groups, including Group 1.1c that are abundant in acidic forest soils, remains unknown. This study aims to unravel their ecological function in such ecosystems using single-cell genomics.
Single-cell sorting was performed on cells extracted from two Scottish pine forest soils in which growth of Group 1.1c Thaumarchaeota has previously been observed with no evidence for ammonia oxidation. Sorting of Group 1.1c cells was achieved by specific cell labelling (using DOPE-FISH) and size selection and the resultant 200 genomes were amplified using multiple displacement amplification. Based on 16S rRNA gene identity, 50 positive thaumarchaeotal genomes were sequenced using Illumina MiSeq technology.
After assembling the sequencing reads cleaned by removal of common contaminants, the size of each partial genomes ranged between 0.02 and 0.3 Mbp, with 60% GC content. The largest contig size, 32 kbp, was obtained in some samples and facilitated annotation of several potential metabolic pathways in these organisms. Among others, several genes involved in the beta oxidation of fatty acids were retrieved, suggesting an important role in fermentation, while no genes involved in ammonia oxidation were recovered. These genomic findings provide interesting and novel hypotheses that remain to be confirmed using soil microcosms. In addition to improving understanding of Group 1.1c thaumarchaeotal function and ecological importance in forest ecosystems, these findings will also inform future enrichment and isolation studies.

18S-RNA-SIP shows a weak correlation between RNA:DNA ratio and production of new ribosomes among soil microorganisms

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Ribosomal RNA (rRNA) is commonly used to characterize active members of mixed microbial communities in ecosystems. Pure culture studies have shown that most microbial rRNA turns over within a few minutes. This observation has been applied to studies of microbial communities in the environment and consequently, soil rRNA is thought to be relatively short lived. However, scientists have recently challenged this paradigm arguing that some rRNA molecules may be “old” and therefore may not reliably indicate actively metabolizing cells. Dormant bacteria, for instance, may contain large amounts of rRNA, and yet not be metabolically active.

We used $H_2{}^{18}O$-RNA-Stable Isotope Probing to characterize newly made rRNA molecules in an arid soil located near Sedona, Arizona, USA. The overall density of labeled soil microbial rRNA was significantly higher than the density of unlabeled rRNA, indicating that large amounts of new ribosomes were produced. In general, most microbial groups were more represented in DNA than RNA sequencing libraries with the exception of a few taxa, including Actinobacteria, Proteobacteria and Crenarcheota which were more prevalent in rRNA than DNA. However, the rRNA of these taxa was not highly labeled with ^{18}O indicating that most of their rRNA was older. In contrast, rRNA of one representative from the phylum Proteobacteria, family Xanthomonadaceae, was highly labeled suggesting production of large amounts of new ribosomes, albeit this bacterium was more abundant in DNA rather than RNA libraries. Because the degree to which rRNA was labeled significantly varied among microbial populations not all ribosomal RNA appeared newly made. Our results therefore question the paradigm that rRNA is a direct indicator of microbial activity.

Bacterial ammonia oxidisers vs. archaeal ammonia oxidisers: who wins in soil, when and why?

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A fundamental issue in microbial ecology is the degree to which phylogeny and function are related, the consequences for relationships between microbial community composition and environmental characteristics and implications for ecosystem function. Bacterial and archaeal ammonia oxidisers provide an interesting model with which to address this issue. These two distinct phylogenetic groups co-exist in most soils and cultivated strains share ecologically important physiological characteristics, despite fundamental differences in cell structure and metabolism. Many correlation-based studies have attempted to determine associations between environmental characteristics and relative abundances of each group. These have been complemented by physiological studies of a limited number of soil isolates and experimental approaches in model systems. These different approaches have led to proposals that two factors, low pH and low ammonium concentration, favour growth of AOA over AOB. This presentation will consider the strength of evidence for these proposals; whether they can be generalised; mechanisms explaining differential effects of pH and ammonia concentration; and predicted effects on ecosystem function.

The communal gene pool in soil

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Horizontal gene transfer in microbial communities in soil leads to the formation of a communal gene pool. The communal gene pool is mobilized by genetic elements such as plasmids, transposons, and viruses. In order to understand how soil microbes respond to stresses caused by various anthropogenic activities, a comprehensive overview of this genetic information becomes crucial,. Our current knowledge of mobile genetic elements (MGEs), and in particular of plasmids, has largely been derived from plasmid genomes obtained through traditional strain cultivation or isolation procedures, like exogenous plasmid isolation. These methods, almost universally, involve the introduction of specific chemical selection biases directed towards antimicrobial resistance or xenobiotic degradation and rarely produce sufficient data to assess mobilome structure on a community-wide scale.
We have developed a novel method for conducting a focused investigation of the communal gene pool of circular genetic elements (metamobilome) present in different natural environments using next generation sequencing. Annotation of the mobilome data from soil revealed that novel genes with no or very little homology to any known genes dominate the gene pool in this particular environment. We also found that environmental stress, such as metal contamination, has a strong effect on the communal gene pool in soil, with a general enrichment of resistance traits including antibiotic resistance genes.
Conjugal plasmids can provide microbes with full complements of new genes and thereby constitute potent vehicles for optimizing microbial fitness through horizontal gene transfer. While broad host range plasmids are known to transfer to diverse hosts in pure culture, the extent of their ability to transfer in complex bacterial communities of e.g. soil has not been comprehensively studied. We have recently developed a culture independent approach to study the potential transfer range of plasmids in natural communities. Our results demonstrate that IncP-type broad host range plasmids have a hitherto unrecognized potential to readily transfer to very diverse bacteria and can, therefore, directly connect large proportions of the communal gene pool in soil.
These finding reinforces the significance of mobile genetic elements in stress adaptation by soil bacteria.

Niche partitioning among N_2O reducing microorganisms and their importance as N_2O sinks

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Nitrous oxide (N_2O) is a major greenhouse gas that is reduced to N_2 by bacteria and archaea harboring the N_2O reductase, which is the only known biotic sink. Recently, we showed that the phylogeny of the *nosZ* gene coding for this enzyme is split into two major clades, with the previously undetected Clade II being equally or more abundant than Clade I in different environments. However, the conditions that select for different N_2O reducing communities, and how such differences effect overall N_2O emissions, are poorly understood. Here, we highlight recent findings based on comparative genomics, experimental work and field studies indicating niche differentiation between the two clades and the crucial role for Clade II in regulating N_2O emissions. A comparison of 652 microbial genomes across 18 phyla illustrated the modularity of the denitrification pathway, as organisms with Clade II *nosZ* were mostly non-denitrifying N_2O reducers while canonical denitrifiers that use N_2O as an intermediate were dominant in clade I. Further, co-occurrence patterns of key denitrification genes were not randomly distributed across taxa or amongst preferred habitats, with *nosZ* occurring more frequently than expected among aquatic organisms. These results underpin the importance of community structure for N_2O emissions. Our experimental research has focused on N_2O reducing communities in sediments and fertilized soils, which are important environments for denitrification. Analysis of N_2O reducing communities in 47 different arable soils across Europe showed that the soil N_2O sink capacity is mostly explained by the abundance and diversity of Clade II, and that niche differentiation or even competitive interactions exist between organisms with either type of N_2O reductase. Interestingly, plant roots selected for denitrifies with a complete denitrification pathway belonging to clade I rather than favoring clade II. Canonical denitrifiers were also identified as the dominant community controlling N_2O reduction in coastal sediments, however specific lineages between and within each *nosZ* clade were associated with different oxygen regimes, again suggesting niche partitioning between the two communities. Altogether, our results indicate that non-denitrifying *nosZ* Clade II organisms are important N_2O sinks and are favored by other environmental factors than N_2O reducers within clade I.

Combining stable isotope labeling experiments and single-cell analysis techniques to detect active microorganisms in soil

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Soils are home to a multitude of our planet's biodiversity yet our understanding of the in situ activities of microorganisms, critical for driving terrestrial nutrient cycles, is limited. One such area that demands a better understanding is the terrestrial carbon (C) cycle. Mineral soils contain a large pool of C, with cellulose as a major constituent. Cellulose degradation can be considered functionally redundant, as members of both bacteria and fungi are capable of this process. However, their exact contributions and ecological niches are still not fully understood. The cellulose-responsive communities and their niche differentiation was investigated via destructive ^{13}C-cellulose-amended soil microcosms supplemented with differing background C and nitrogen, followed by a multidisciplinary approach encompassing microbial respiration, extracellular enzymes, ^{13}C-PLFA-SIP and ^{13}C-DNA-SIP. Analysis of the ^{13}C-enriched PLFA and DNA revealed distinct bacterial and fungal communities across the treatments. Using this community-based analysis, we are currently identifying target groups to further investigate their activity at the single-cell level by combining FISH and high-resolution secondary ion mass spectrometry (NanoSIMS). Until now, single-cell approaches have found limited application in soils presumably due to the dispersal of microbial cells in a large background of particles. As such, we developed a soil sample preparation procedure to efficiently retrieve cell extracts by combining cell detachment with separation of cells and soil particles followed by cell concentration. The procedure was evaluated by examining its influence on cell recoveries and microbial community composition. This approach allowed the single-cell analysis of cellulose-responsive soil microorganisms by NanoSIMS. The same procedure is also applicable for detecting stable isotope-enriched soil microorganisms by Raman microspectroscopy, assessed via microcosm incubations with a ^{13}C-labeled C source and deuterium oxide (D_2O, a general activity marker). This soil sample preparation procedure enables single-cell analysis using NanoSIMS and Raman microspectroscopy not only of active cellulose-responsive soil microorganisms, but also of microorganisms actively involved in other targeted processes.

The trajectories of denitrifier structure and function demonstrate remarkable differences in soil with a legacy of anoxic spells versus constant oxic conditions

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Denitrification is an important environmental sink for nitrate, and a main source of N_2O. One key environmental controller of denitrification is O_2. This study tested how the legacy of O2 availability in soil influences denitrification kinetics and denitrifier community structure. We hypothesized that denitrifying communities that experience frequent anoxia will readily express the denitrification reductases and demonstrate faster denitrification rates than those living under steady, oxic conditions, but short anoxic spells will disfavor the expression of N_2O reductase since this enzyme is supposedly more sensitive to O_2 than the other denitrification reductases. Grassland soil samples were exposed to either frequent, short anoxic spells; long anoxic spells; or fully oxic conditions over a 30 day period. All microcosms were then incubated anoxically and the kinetics of NO, N_2O and N_2 were determined. Samples were taken during the anoxic incubation for metagenomic and metatranscriptomic analyses. Significant functional differences were seen among the three treatments, especially in regards to N_2O accumulation. Surprisingly, the oxic treatment had the fastest denitrification rate, completing reduction of nitrate to N_2 in 25 hrs, with the lowest accumulation of intermediates, while the short anoxic treatment took over 100 hrs to complete to N_2 with the largest intermediate accumulation. In concurrence with the functional data, the oxic treatment demonstrated the highest abundance of genes encoding denitrification reductases compared to the other treatments, while the short anoxic samples showed the least abundance of genes encoding N_2O reductase. Analysis of the metatranscriptomic data with time reveals that the oxic samples increased in abundance of genes encoding denitrification reductases while the long anoxic decreased, not necessarily correlating with the gas kinetics. The most highly expressed N_2O reductase genes in the long anoxic treatment vary in taxonomic composition compared to the oxic and short anoxic treatments, which may explain the surprising suppression of its denitrification kinetics. These results suggest that the exposure to frequent anoxic spells can slow denitrification and result in a higher release of denitrification intermediates, which may have global implications to climate change.

The soil fungal-bacterial interactome - Mechanisms of interaction, with special emphasis on *Burkholderia terrae*

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Burkholderia terrae strains BS001 and BS110 are interactive with the ectomycorrhizal fungus *Laccaria proxima*, and with *Lyophyllum* sp. strain Karsten. Strain BS001 produces biofilms on fungal hosts and sequesters glycerol from it. It can also castrate its host, inhibiting primordium setting. BS001 has broad comigration capacity with six of nine fungi belonging to the ascomycota and basidiomycota. The 11.5-Mb genome contains a range of genes for mycosphere-relevant functions. Regions of genomic plasticity (RGP) included the 70,422 kb RGP79, containing a type-4 secretion system and other plasmid-type traits. Biofilm formation genes and type-2, type-3 and type-6 secretion systems are also present. Using mutation analysis, the type-3 secretion system was found to be important for interaction with the fungus. *B. terrae* BS001 can utilize numerous carbonaceous compounds, e.g. glycerol, methylglyoxal, fatty acids, sugars, amino acids. The genome is a legacy of a versatile biphasic lifestyle. Transcriptome analyses revealed BS001 modulates the expression of key genetic circuits as a response to a soil–mimicking environment and fungal hyphae. The stationary–phase sigma factor *RpoS*, as well as genes under its control, were expressed to a large extent across treatments. Strain BS001 upregulated several chemotaxis–related genes, as well as a gene encoding a SET-domain-containing (secreted effector) protein. Five genes potentially involved in oxidative stress responses were also highly upregulated. Being in a stress–dominated state, BS001 showed early and late responses to *L*. sp. strain Karsten, i.e. dynamically–changing chemotaxis, potential metabolic signalling and an oxidative stress response.

Arbuscular mycorrhizal fungi proliferate in patches of soil enriched with N-containing organic compounds

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Large fraction of mineral nutrients in natural soil environments is recycled from complex and heterogeneously distributed organic sources, explored by roots and associated mycorrhizal fungi. Responses of arbuscular mycorrhizal (AM) hyphal networks to soil organic patches of different qualities are, however, still little understood. We conducted a multiple choice experiment addressing hyphal responses to eight different patch qualities by two functionally different AM fungal species (*Rhizophagus irregularis* and *Claroideoglomus claroideum*) in association with *Medicago truncatula*. Hyphal colonization of the patches was assessed microscopically and by quantitative real-time PCR with taxon-specific markers, and the prokaryotic and fungal communities in the patches were profiled by the 454-sequencing.
Hyphae of both AM fungi responded positively to nitrogen (N)-containing organic amendments (i.e., chitin, DNA, albumin, and clover biomass), while no responses were recorded for cellulose, phytate or inorganic phosphate amendments as compared to the non-amended soil patch. Abundance of several prokaryotes incl. *Nitrosospira* sp. (an ammonium oxidizer) correlated positively with the hyphal responses of *R. irregularis* to the soil amendments. We demonstrate, using two independent analytical approaches, a consistent stimulation of AM hyphal networks by different N-containing organic soil amendments. Exact mechanism of this effect remains yet to be identified.

Do microbial carbon use efficiency (CUE) and the mean residence time (MRT) of microbial biomass depend on soil stoichiometry?

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The ratios of bioavailable elements in soils hardly ever meet the nutritional demands of soil microbial communities. Yet, the microbial biomass stoichiometry is relatively constant. To maintain their biomass stoichiometry, microbial communities can adjust their carbon use efficiency (CUE) and the mean residence time (MRT) of their biomass to the ratios of available elements. Microbial CUE is usually defined as the organic C taken up that is allocated to growth. So far, it is not well understood how microorganisms adjust their CUE and the MRT of their biomass to ratios of available elements in soils due to a lack of suitable methodological approaches. Microbial CUE has been measured by determining the incorporation and respiration of C from specific ^{13}C-labeled substrates. However, this approach confounds microbial CUE with the specific use efficiency of a given substrate.
Recently, we developed a method to determine both the microbial CUE and the MRT of the microbial biomass C independently of substrate addition. The new method is based on labeling of microbial genomic DNA with ^{18}O-H_2O. Since genomic DNA is only synthesized when cells are dividing, the incorporation of the ^{18}O-label into genomic DNA can be used to calculate the microbial growth rate. Based on the growth rate and the respiration rate measured concurrently, the microbial CUE is estimated. Moreover, the method can be used to assess the MRT of the microbial biomass C in soil. To calculate microbial MRT, the microbial biomass C is divided by the microbial growth rate.
Here we will show first results on the microbial CUE and the MRT of the microbial biomass in temperate soil profiles and in grassland soils of a long-term fertilization experiment. The CUE determined based on ^{18}O-labeling was consistently lower than the CUE estimated by ^{13}C labeling. The microbial CUE in organic layers of forest soils was significantly higher than in the mineral soil, where it amounted to about 30%. In fertilized grassland and cropland soils, the microbial CUE was elevated compared to the mineral horizons of the forest soils. The MRT of the microbial biomass C in the forest soils increased strongly with depth from 8-44 days in the organic layer to 253-294 days in the B horizon. We will discuss the dependence of the microbial CUE and MRT of the microbial biomass C on ratios of bioavailable C, nitrogen and phosphorus concentrations.

Regulation of nitrite concentrations in acidic and neutral pH soils by a combination of chemistry and complex bacterial community regulation: A study of kinetics and transcriptomics

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Our previous studies on agricultural peat soils from a long-term liming experiment in Norway showed that under anoxic conditions, NO_2- concentrations were consistently low in low pH soils, but accumulated to mM concentrations in neutral pH soils. Inversely, low pH soils produced large amounts of gaseous NO and N_2O before reduction to N_2, while neutral pH soils accumulated lower quantities of NO and N_2O, and produced N_2 immediately upon anaerobisation. Although the lack of NO_2- accretion in acidic soils has largely been attributed to the non-biological reduction of NO2- to gaseous N-oxides, i.e. chemodenitrification, some form of biological control has not been completely excluded. Our primary focus in this study is to differentiate between chemical and biological denitrification. Moreover, we wanted to identify differences in denitrifier community composition and which genes that are expressed during transition from oxic to anoxic respiration in soils with contrasting pH.
We modelled the nitrite kinetics of sterilised soils, and confirmed that chemodenitrification rates are highest in acidic soils (pH 3.8), and negligible in near-neutral pH soils (pH 6.8). Taking chemodenitrification into account when calculating gaseous N-oxide production rate, we were able to establish that NO_2- reductase activities in both neutral and acidic soils are comparable, and verified that biological reduction of NO_2- is significant in acidic environments. Following this, we investigated the transcription patterns of genes related to nitrogen-cycling in peat soils of different pH (pH 3.80, 5.73 and 6.80). To associate denitrification kinetics with transcription patterns, we monitored the accumulation and consumption of NO_2-, NO, N_2O and N_2 following NO_3- addition, and sampled soils frequently for DNA and mRNA analysis. To overcome the co-extraction of inhibitors in our highly humified peat samples, we optimised nucleic acid extraction and were able to ensure low inhibitor interference with downstream processes such as quantification and sequencing. The qPCR results suggest temporally distinct transcription patterns of denitrification-associated genes controlled by the concentrations of NO_2-, O_2 and NO in our peat soils. To further examine the regulation, extracted DNA and RNA sampled at different time points are currently undergoing meta-genomic/-transcriptomic analysis using Illumina HiSeq technology.

AM fungal hyphae exudates can prime a bacterium mediated phytate mineralization in hyphosphere

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A main subject in ecology is to understand how cooperative strategies evolve and are maintained in species networks. Here, we focus on the three-partner relationship between plants, arbuscular mycorrhizal (AM) fungi and hyphosphere bacteria to ask if the interaction between AM fungi and bacteria can pay back the resource (phosphorus here) to host plants by consuming the plants derived carbon (C). Two microcosm experiments which separate the plant roots and mycorrhizal hyphae and bacteria were conducted to demonstrate the direct effects of hyphal exudates on the growth and activity of bacteria in organic phosphorus (P) mobilization and the direct effects of bacteria on the growth and activity of the AM fungus in P uptake which were transferred to plants. Results showed that AM fungus released substantial C to the environment, triggering bacterial growth and stimulating their activity on organic P mineralization and turnover. Analysis with ^{13}C -DNA stable isotope probing was used to track the C flow through hyphae exudates to hyphosphere bacteria community. We found *Pseudomonas alcaligenes* which has been previously identified to mineralize phytin was labeled with ^{13}C derived from maize photosynthate via extraradical mycelium of *R.irregularis*, indicating the phosphorus solublizing bacteria strain used hyphae exudates. The bacteria enhanced hyphal proliferation by which the fungus have more access to available P. Our results suggest that AM fungi and bacteria share photosynthates of plant, and as reciprocation, the AM fungi-bacteria interaction repays the plant with P by jointly mobilizing soil organic P.

Alien escape: impacts of bacterial invasions on soil microbial communities

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There has recently been a surge of literature examining microbial invasions into a variety of environments. These studies often include a component of biological diversity as a major factor determining an invader's fate, mostly through resource competition. Yet, few studies have attempted to assess any potential impacts and consequences of invasions on soil microbial communities. In this study, we experimentally created diversity gradients of soil microbial communities that were transiently invaded with a non-pathogenic derivative of *Escherichia coli* O157:H7. We assessed the impacts of invasion by measuring four community parameters: (i) alpha diversity, (ii) taxonomic structure, (iii) niche breadth, and (iv) niche structure. The former two parameters were assed via 454 pyrosequencing for 16S rRNA genes of each community. Niche breadth, defined as the number of different resources used, and niche structure, defined as the patterns of resource use, were assessed by measuring the metabolic activity of soil microbial communities across an array of 71 carbon sources. Invasion resulted in increases of alpha diversity and niche breadth that were respectively paired with shifts in taxonomic and niche structures. These impacts also scaled with microbial community diversity where less diverse communities experienced greater impacts. Furthermore, our results strongly suggest that the invasion-induced shifts in the taxonomic and niche structures of the soil community were driven by competition for resources between the resident community members and the invader. The main process is likely the selection of resident bacterial taxa with functional traits, allowing them to exploit niches that were metabolically unusable by the invader. Overall, these data show that the invader, even ill-adapted to the soil and leading only to a transient invasion, competed for resources with microbial residents and steered the community preferentially towards niches that the invader could not occupy. This demonstrates that even transient invasions have lasting effects on native communities, which can be understood in light of competition for resources. In general, our data demonstrates that understanding the legacy of, even unsuccessful, microbial invasions, is crucial for estimating their fate and impact – as overlooked issue in many practical applications where microbial invasions are key, such as the efficacy of probiotics and the survival of biocontrol and biofertilizing agents.

Microbiology of soil primary organo-mineral complexes and particulate organic matter

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Most soil microorganisms live attached to or in close vicinity of particle surfaces. In intact soil, the differently sized primary organo-mineral complexes (sand, coarse silt, fine silt, clay) and particulate organic matter (POM) constitute building blocks of a highly heterogeneous and hierarchical system of micro- and macro-aggregates. The differently sized primary particle fractions (PSF) differ in their mineralogical composition and specific chemical sorption capacities, and, thus, should select for structurally and functionally different communities at their surfaces. To test this hypothesis, PSF of soil organic carbon variants from a long-term fertilization experiment (Askov, Denmark), including alternatively mineral fertilizer or manure, were analyzed for their microbial diversity and their capacity for mineralizing two organic pollutants (phenol and 2,4-dichlorophenol, DCP). Fractionation of PSF with their associated microbial communities was achieved by wet-sieving including a mild-ultrasonication and centrifugation steps. Massive parallel sequencing of V4 16S rRNA gene amplicons from non-fractionated soil and PSF revealed that presence of approx. 96,000 different OTU (operational taxonomic units). About 4% bulk soil sequences of mainly rare OTU were not found in PSF, while approximately the same amount of rare OTU were only detected with the different PSF. The coarser PSF were the preferred habitat of Proteobacteria, while Acidobacteria and Firmicutes appeared to prefer smaller PSF. Gemmatimonadetes were most abundant in coarse silt, Actinobacteria in fine silt, and Planctomycetes in clay. Quantitative PCR revealed that manure addition increased the abundance of Bacteria, Archaea and Fungi, with coarse silt being most responsive. The manuring effect declined with decreasing particle size. All PSF actively mineralized phenol, but only clay mineralized DCP. Phenol mineralization rates correlated negatively with particle size, except for sand/POM. Overall, these results indicate that soil primary particles and POM select for structurally and functionally distinct microbial community members - many of them potentially overlooked in common studies based on non-fractionated soil only.

Links between patterns of soil microbial diversity and sustainable soils

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Soil-borne microbial diversity is vast and critical to the functioning of agro-ecosystems. With the increased pressures being placed on soils, soil degradation has become a major global problem at a time that increased food production is required to feed and an ever-increasing human population. Thus, it remains a grand challenge to design and implement sustainable agricultural practices that provide high yield and food security while at the same time reducing artificial fertilizer and pesticide inputs and maintaining soil fertility. Such future strategies will require more informed and integrated reliance on the inherent functional properties of soil-borne (microbial) communities, including those involved in nutrient acquisition, plant defense and regulation of greenhouse gas emissions. I will present a number of fundamental properties related to the drivers of microbial diversity in soils, and then relate such patterns to the procurement of ecosystem functions required for sustainable agricultural practices and the maintenance of soil fertility. Special focus will be paid to the need to understand the interactions that drive and maintain microbial diversity and community structure and experimental approaches designed to zoom in on active players in soil nutrient cycling and plant growth promotion.

The diversity and functions of protists in soil: problems and progress

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Protists are at the base of soil food webs, connecting the flows of energy from plants through bacteria and fungi to higher trophic levels in the soil food web. Laboratory experiments demonstrated strong effects of protists on plant growth, but despite their functional importance, our knowledge on the community composition of protists in soil is still highly contradictory. Despite environmental sequencing studies based on the SSU rRNA gene have revealed a huge diversity of previously unknown protists, new biases are being introduced by molecular techniques, obscuring the true protist diversity in soils. Fundamental problems are created by (i) the lack of SSU rRNA reference sequences for major protist clades, (ii) vast mislabelled sequences in public databases, and (iii) the enormous phylogenetic diversity of protist taxa, all commonly resulting in erroneous species assignments. Further biases are introduced by the PCR step that usually precedes high-throughput sequencing (HTS) of SSU rRNA gene studies. “General” eukaryotic primers are often applied to decipher the community structure of protists, but these primers are in fact far from being truly universal. While some taxa within this subset will be overrepresented due to preferential PCR amplification, other (dominant) taxa are entirely lacking, resulting in a strongly biased view of the protist community in soils. I will present some recent data on progress in molecular methods to describe the protist diversity in soils, and on linkages of the protist micro-food web to the root microbiome and carbon fluxes from plant roots into the soil.

What does the microbiome tells us about life in the plant root zone?

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Studies of plant root-associated microbiomes have revealed high diversity along with signature host species-specific and niche-specific associations. The functional implications (ecosystem, plant health) of these associations remain unexplained, however. We have sampled soil and roots of wheat and cucumber plants growing in identical soil conditions. The soil and plant-associated bacterial community composition was determined using 16S rRNA sequencing. In addition, large-scale metagenome and metatranscriptome sequencing was used to characterize community structure, functional potential and expressed physiological activity. A large database of approximately 2.4 million non-redundant ORFs was assembled from soil and root genomic DNA. The diversity of each habitat's microbes varied widely between niches and between plant species. Based on metagenomic data, the functional characteristics of soil versus root bacteria deviate dramatically. Soil-to-root functional variations were particularly apparent in specific pathways including secretion systems, motility, ABC transporters, two-component systems, lipopolysaccharide metabolism and carbohydrate metabolism. In root-associated samples, the metagenomes were functionally similar between the different plant species, despite significant differences in community composition, suggesting the recruitment of root community was governed by a basic suite of genes, common to host-colonizing bacteria. Conversely, transcriptional profiles of root-associated communities were non-redundant. For example, high relative expression levels of nitric-oxide reductase genes characterized wheat root communities, while in cucumber root communities, expression of these genes was negligible. An opposite trend was found with respect to catalase and pectinases genes expression. These differences outline the structural as well as functional implication of host-microbe interactions that are crucial for plant growth, health and development in soil.

Soil Volatile Organic Compounds: Microbial Competition Tools with High Potential for Control of Root-Infecting Pathogens

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Production of volatile organic compounds (VOCs) has been demonstrated for many bacteria and fungi isolated from soils. Several of these compounds have antimicrobial activity and especially the inhibition of root-infecting pathogens has drawn attention because of unexplored possibilities for control of crop diseases. However, so far the production and functioning of microbial volatiles in agricultural soils and the relation with microbial community composition and diversity has not been studied extensively.
In this presentation we integrate results of several of our recent studies on the relationship between VOC production, composition and diversity of soil bacterial communities and suppression of fungal plant pathogens. The major findings are that (1) Production of pathogen-inhibiting VOCs in agricultural soils is a general phenomenon, (2) Inhibition of fungal pathogens by VOCs emitted from soil samples is correlated with disease suppression (crop bio-assays), (3) Bacterial community composition and – diversity are influencing VOC production, (4) Shifts in the bacterial community composition and reduction of diversity can lead to reduction or even loss of the production of suppressive VOCs.
The results indicate that VOCs play an important role in the ecology of soil micro-organisms. A better insight in this aspect of soil microbial ecology has strong potential to steer the natural buffering capacity of soils against soil-borne plant diseases.

Evolution and Distribution Patterns of Nitrite Reductase (*nirK/nirS*) in Soil Metagenomes suggest Functional Differences Between Lineages

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Denitrification, the stepwise reduction of nitrate to nitrogen gas, is a pathway of critical importance within the nitrogen cycle, being a key driver of nitrogen loss in agricultural soils as well as emission of the greenhouse gas nitrous oxide. This functional trait is widespread in bacteria, and 16SrRNA surveys alone are not enough to infer its presence in microbial communities. The reduction of nitrite to nitric oxide can be catalysed by one of two non-homologous nitrite reductases, encoded by the genes *nirS* and *nirK*. This step is considered to be the defining step of canonical denitrification, thus the presence of these genes have been extensively used as indicators of denitrifiers in natural environments. Recent studies that have detected novel structural variants and lineages, along with known primer biases, call for a revision on our knowledge on *nirK* and *nirS* genes in natural environments. Using full length sequences from complete genomes, we resolved robust phylogenies for both genes with nine lineages for *nirS* and 13 for *nirK* that differ in sequence, protein structure and both taxonomy and lifestyle of the harboring organism. Inspection of currently available primers show that existing primer sets cover only a small proportion of the extant diversity of either gene, and were not specific to different lineages. We further performed an exhaustive search of homologues for each lineage in 1093 metagenomes from different habitats, including 98 soil metagenomes, and confirmed differences in abundance and distribution patterns of the lineages across habitats. In soils, *nirK* homologues were more in general more abundant than *nirS* homologues, except in permafrost soils. The *nirS* lineage corresponding to canonical denitrifiers dominated the *nirS* reads in soil metagenomes, whereas four uncharacterized lineages of *nirK* accounted for 60% of all *nirK* homologues. The ecology and physiology of the organisms in each lineage, together with the distributional patterns of these lineages in specific habitats, suggest distinct ecological roles for different lineages. This calls for a reevaluation of the inferred dominance of canonical denitrification in natural environments, and a reconsideration of the functional roles of denitrification. Finally, we propose a set of lineage-specific primers to evaluate different aspects of nitrite-reducers communities, as well as a phylogenomic-informed classification system for environmental sequences.

The diversity of free-living, non-diazotrophic *Bradyrhizobium* from contrasting soils

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The bacterial genus *Bradyrhizobium* is biologically important within soils, with different representatives found to perform a wide range of biochemical functions including photosynthesis, nitrogen fixation through symbioses, denitrification and aromatic compound degradation. Some phenotypes have been found to be non-symbiotic and have either never acquired, or lost the ability to form nodules and are assumed to have different roles in soil. Bradyrhizobium-like species have been shown by many metagenomic studies on soil to be one of the most abundant and active groups. The Highfield experiment at Rothamsted provides an opportunity to study the impact of plants on microbial communities as it has three long-term contrasting regimes; permanent grassland, arable and bare fallow (devoid of plants). The bare fallow plots have been maintained in this way since 1959 and this has resulted in a significant reduction in soil carbon and microbial biomass. The relative abundance of *Bradyrhizobium* in bare fallow soil demonstrates that they are adapted to survive in the absence of plants although the increased abundance in grassland soil indicates that some may associate with plants.
A culture collection was created with isolates obtained from contrasting soil types: grassland; arable and bare fallow; woodland; and gorse (*Ulex europeaus*) and broom (*Cystisus scoparius*) root nodules from scrubland. One grassland and one bare fallow *Bradyrhizobium* isolate were chosen to have their genome sequenced using Ion Torrent and Illumina sequencing platforms. The genomes were assembled using SOAPdenovo2 and manually curated using Geneious. The genomes show a distinct difference in size. Differences between the genomes have been assessed in order to establish the different functions these isolates are able to perform to give clues to their role in the community. The phylogeny and functional diversity of the culture collection have been explored using PCR and carbon metabolism assays to determine whether land management impacts the genetic and metabolism potential of this group.
The next steps to help determine the role of this genus in the Highfield soils and in its wider ecosystem context are to combine the phylogeny and functional diversity analyses to discover patterns within the *Bradyrhizobium* culture collection and to use the genomes to mine the metagenome and direct future experiments.

Biodiversity of synthetic microbial communities determines disease suppression

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Synthetic microbial communities are increasingly used in biotechnological processes, where they combine properties unreachable by a single species. One function of particular importance is pathogen suppression, where beneficial microbes may cure infections inaccessible to chemical treatments. Plant diseases are a major threat to global food security and application of beneficial microbes may prove efficient at suppressing diseases in a sustainable way. Here we fight *Ralstonia* wilt with synthetic communities containing one to eight probiotic *Pseudomonas* spp. We added the bacteria to tomato growing in a natural soil infested with pathogens. We show that the effect of the community increases with the number of introduced species. Single-species inocula conferred a partial protection, yet eight-strains mixtures reached a complete disease suppression and reduced pathogen density by a factor 10 compared to the best single-strain communities. We relate these effects to an increased antagonistic activity and a higher competitiveness of multispecies communities. This study indicates that biodiversity-ecosystem functioning relationships can be used to develop highly functional synthetic communities to suppress diseases and replace pesticides.

An indicator for disease suppression: linking soil chemistry to microbiology using dissolved organic carbon fractionation

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Soil organic matter (SOM) is a major component of soil quality, contributing to physical, chemical and biological properties of the soil. Incorporation of organic matter may stimulate the soil microflora, increase microbial activity and biodiversity and thus enhance suppression of soil borne pathogens by competition, predation and/or specific antagonism.
In a field experiment, a mixture of peat (95%) and manure (5%) was incorporated, creating three levels of SOM: 10, 20 and 30 g kg-1. Soil samples were analysed for a range of physical, chemical and biological soil parameters. Samples were also tested in bioassays for disease suppression against *Pythium intermedium*, *Rhizoctonia solani* and *Melodogyne* hapla using Hyacinth, Tulip and Lettuce respectively as test crops. SOM levels had no effect on sprout infection by *Rhizoctonia*. For both *Pythium* and *Melodogyna*, less disease symptoms were observed at higher SOM contents. It was concluded that higher SOM induced disease suppression against these two pathogens and that this suppression was mainly due to biological activity since in sterilized soil little or no suppression occurred. Multivariate analyses and multiple regressions obtained with model selection, showed positive correlation between disease suppression, SOM and hot water extractable carbon (HWC). Based on these results, SOM and soluble organic carbon have been proposed as indicators for disease suppression. Fractionation of soil dissolved organic carbon (DOC) may even give more detailed information on SOM quality as a substrate for the soil microflora and subsequent antagonistic activities. In another field trial, four different types of organic inputs were incorporated in the soil: compost, coconut fibre, cacao shells and biochar. The raw materials differed significantly in DOC concentrations, ranging from 4 mg kg^{-1} to more than 9000 mg kg^{-1}. DOC was further qualified by fractionation into pools of humic acids, fulvic acids, hydrophobic neutrals, and hydrophilic compounds. After amendment, soil physical, chemical and biological parameters were measured, including total DOC, DOC fractions and disease suppression. Statistical analyses showed that variation in disease suppression was best explained with models including total DOC, humic acids and fulvic acids, rather than SOM or HWC. Based on these results, qualification of DOC pools through fractionation may be an informative tool in predicting soil functional processes such as disease suppression.

Protozoa induce soil suppressiveness against *Fusarium* wilt

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Soil borne diseases are responsible for important harvest losses worldwide and are a threat to global food safety. Some soils are naturally suppressive to pathogens and there is a growing corpus of evidence that microbiota play an essential role in shaping suppressiveness. Most research has focused on bacterial communities. Here we show that protozoa are an essential component of soil suppressiveness. Soil protozoa are major consumers of bacteria and have a strong impact on the structure and function of soil microbial communities. Here we show that addition of low amount of protozoa to a natural soil are sufficient to induce suppressiveness to *Fusarium oxysporum*, a major disease affecting several plant families. We saw faba beans in a natural soil infested with pathogens and inoculated the plants with three different protozoa. Protozoa addition reduced pathogen load to a factor 10'000 in soil and completely suppressed disease symptoms. We conclude that protozoa should be taken into account when investigating pathogen suppression by the soil microbiome.

Life strategy and life cycle of Tuber melanosporum: a pioneer hermaphrodite with high spore bank and functional dioecism

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The ectomycorrhizal Black Truffle (*Tuber melanosporum* Vittad.) spontaneously grows in open woodlands, either spontaneous (= natural) or planted. Our researches in Southern France questioned its biology and ecology of in natural or planted environments, in order to understand some aspects of Black Truffle vegetative and reproductive development potentially limiting the production of this prized fungus.
Barcoding of ectomycorrhizal communities confirms that the Black Truffle is a pioneer, successional symbiont, which is quite specific to *Quercus* spp. in the investigated shrub ecosystems with sparse trees preceding canopy closure, and which disappears from mature forests. It forms ectomycorrhizae, but we found molecular evidence that roots from various herbaceous plant species, arbuscular mycorrhizal or non-mycorrhizal, also harbor Black Truffle. It likely develops as a root endophyte, although the exact interaction remains unclear.
Although hermaphrodite, the Black Truffle is self-sterile due to incompatibilities controlled by a mating-type gene with two alleles (+ and -): its meiotic spores are formed in ascocarps supported by one (maternal) parent after fertilization by a second (paternal) parent. We confirm statistically the previously suspected existence, both in natural and planted populations, of segregated patches of maternal individuals with identical mating types (i.e. exclusively + or -), which colonize ectomycorrhizal and herbaceous hosts. Microsatellite analyses also confirm the previously observed strong isolation by distance among maternal individuals, probably due to local deposition of spores from same ascocarp(s) by animal dispersers, and to an important soil spore bank.
Diploid zygotic ascocarps are highly inbred (although to a lesser extent in planted truffle-grounds), supporting very limited spatial dispersal of paternal gametes. Since paternal mycelia were never found on investigated roots, we hypothesize a contribution of germlings from the spore bank. We also show that, due to segregated patches of mating types, individuals from one of the mating types can locally behave as maternal contributors, while individuals from the other mating type are restricted to a paternal contribution (and likely, a limited development), resulting in a functional dioecism.
We discuss these results in the framework of current practices in truffle-grounds, and propose the Black Truffle as a default paradigm for sexual ectomycorrhizal Ascomycota.

Arbuscular mycorrhizal fungal communities: global and local patterns and their potential drivers

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Mycorrhizal fungi are important components of the soil microbial community, with functions in ecosystem productivity and diversity. Arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota) colonize the roots of about 80% of terrestrial plants, facilitating mineral nutrient uptake from soil in exchange for plant-assimilated carbon. While the distribution of plant species in different regions and along environmental gradients is relatively well described, the biogeography and community ecology of microorganisms, like AM fungi, is at its earliest days. Due to the cryptic lifestyle of AM fungi, knowledge about the variation of global and local patterns of species distribution remains scarce, although new information is gradually accumulating due to the rapid development of molecular tools which makes possible to study those cryptic but essential plant symbionts.

Based on morphological characteristics, described global diversity of AM fungi is relatively low (ca 250 spp.). Since advent of molecular tools, the diversity estimates have increased due to the accumulation of environmental DNA based taxa. However, even those increased estimates of global diversity (ca. 350-3500 spp.) remain modest compared with their host plant taxon pool (ca 200 000 spp). Therefore, it has been long assumed that AM fungi are not host specific and as many other microorganisms exhibit global distribution. Recent studies have shown, however, that there is evidence of host preference, as well as plants' selective allocation of carbohydrates to different fungal symbionts. Recent studies of variation of AM fungal communities have shown that despite their global distribution – more than 90% of taxa have recorded on multiple continents - these fungi are not equally distributed on landscapes and significant variation between local AM fungal communities exists. The main local drivers are edaphic conditions, biotic interactions (host plants, other soil fungi) and type of land use.

In this talk we shall present and discuss results about AM fungal community surveys from global (across continents), regional (across ecosystems) to local (a community) scale. We identify possible mechanisms underlying AM fungal distribution at different scales and define gaps in our knowledge.

From mutualism to antagonism: iron acquisition during soil microbial interactions

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Iron is a limiting nutrient in soil because of its low bioavailability. Microorganisms have developed different ways to capture iron in soil, among which the production of iron chelating molecules called « siderophores ».
Pseudomonas fluorescens BBc6R8 is a Mycorrhiza Helper Bacterium, so called because of its ability to stimulate the formation of ectomycorrhizae by Laccaria bicolor on tree roots. In soil, the bacterium interacts with fungal hyphae but also with other bacteria with which it can get to compete for nutrients, among which iron. In this work, we show that the bacterium uses different strategies to acquire iron when interacting with different microorganisms. It either produces its own siderophores, inhibit the production of other's siderophores or it can pirate the siderophores produced by other microorganisms such as *Streptomyces ambofaciens*. We show that this can lead to shift the interactions from mutualistic to antagonist depending on the availability of iron. Indeed, when iron is not limiting *P. fluorescens* Bc6R8 stimulates the development of *L. bicolor* S238N. However, it completely inhibits the growth of the fungus on media depleted with iron. This negative effect is abolished in *P. fluorescens* mutants unable to produce pyoverdine siderophores. These pyoverdine mutants even stimulate better than the wild type the growth of the fungus. Altogether, our data indicate that the bacterial strain produces different responses depending on the biotic and abiotic context and that it cannot always be considered as a "helper" bacterium. In addition, we demonstrate that this behaviour is not restricted to the model strain *P. fluorescens* BBc6R8 but is widely shared among P. fluorescens.

Can arbuscular mycorrhizal fungi drive vascular plant secondary succession in alvar grasslands?

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Mycorrhizal symbiosis play a key role in plant community assembly over successional processes, having a complex array of interactions with different plant species. Over time, interactions between plants and mycorrhizal fungi are expected to boost mycorrhizal over non-mycorrhizal plants. As a consequence, mycorrhizal plants will become dominant in the ecosystem. Differences in colonization and persistence strategies among plants and arbuscular mycorrhizal fungi (AMF) may be responsible for vegetation dynamics. Several hypotheses have been proposed in order to explain whether changes in AMF (Driver Hypothesis), plants (Passenger Hypothesis) or abiotic conditions (Habitat Hypothesis) drive successions. Such hypotheses rely on assumptions about the existence and direction of symbiont specificity and the order of arrival of symbiosis partners (i.e. first to arrive will drive succession). As far as we know, these assumptions have not been tested to explain the relationships of plant and AMF communities in semi-natural environments. The aims of this study were: (1) to describe plant and AMF communities across a successional trajectory, (2) to assess to what extent AMF and plant communities co-vary, and (3) to determine the prevalent hypothesis describing the co-variation of plant and AMF communities in a secondary succession of alvar grasslands in Western Estonia. We selected sites after 20 and 50 years of gravel pit abandonment and undisturbed alvar grasslands. The abundance and diversity of AMF in soil and roots and plants above ground were analysed using ordination techniques and generalised linear mixed models. Abundance of plants increased, while AMF remained constant over time. Richness of both of them rose in parallel along the succession. This is suggesting that AMF can be abundant at low plant cover, although there may be a certain degree of symbiont specificity. Diversity of plants is better explained by AMF communities than vice versa, suggesting that plant community depends on fungal community composition. AMF exhibited a higher proportion of species pool completeness than plants suggesting that AMF were less dispersal limited than plants. In sum, our results point towards Driver Hypothesis operated in the study secondary succession. The implication of our results is that plant species compatible with superior colonizing ability AMF get advantage to establish unless AMF with superior persistence ability replace early arrival AMF.

Impact of the extraction method for nucleic acids on microbial community composition as assessed by amplicon sequencing

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Soils are one of the most complex environments on earth. Besides their intricate architecture compromising soil organic and inorganic matter they harbor a large diversity of microorganisms. To identify these microorganisms a number of DNA extraction methods have been developed over the last decades, typically involving an initial bead-beating step to release nucleic acids from the soil matrix. Different DNA extraction methods were shown to create a bias regarding the amount and completeness of nucleic acids extracted. The issue of sample preparation received even more attention as it was recently shown that the DNA extraction kits themselves can be already contaminated with microbes.
Previous studies assessing the bias introduced by extraction methods mostly focused on differences between the methods and the question if a core microbiome can be identified that is detected by each extraction protocol and thus independent of the extraction method was rarely addressed.
To systematically assess the impact of different DNA extraction protocols when studying microbial communities in soil we used six well-established and commonly used DNA extraction protocols and isolated DNA from five different soils across Europe. The soils analyzed differed in their chemical composition as well as their texture. After DNA extraction, microbial community composition and diversity was assessed by amplicon sequencing targeting the 16S rRNA gene for bacteria and the ITS region for eukaryotes.
Our results indicate that for each soil a specific core microbiome can be identified independent of the extraction protocol at an OTU level of 97 % similarity. This core microbiome contained, depending on the soil, between 40 – 48% of OTUs that were detected on average by one extraction method. Comparing the core microbiomes of the different soils, we detected pronounced differences as expected by the different soils analyzed. Accordingly, diversity analyses showed very similar results in α- and β-diversity for the different soils and no significant influence of the DNA extraction method used.
Overall our study confirmed that each extraction protocol does introduce a bias into the analysis – however a large number of OTUs were found independent of the extraction protocol, indicating a comparability of data sets to a certain extent even if different extraction protocols were used.

Fungal communities across a mixed temperate forest: Are local site properties the most influential or does whose your neighbour matter?

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Background/Question/Methods
Despite the obstacles to obtaining a clear picture fungal community ecology, their importance in ecosystem processes has long been recognized. This diverse group of organisms that can grow as single-cells to long-lived kilometer-wide hyphal networks are well-studied for their beneficial roles such as their association with plant roots and their important role in nutrient turnover (partially due to their unique ability to break-down lignin), as well as for their detrimental pathogenic roles such as that of the potato fungus causing the "Great Famine" in Ireland. Recognizing these important roles of fungi in ecosystem processes, we are now rapidly expanding our knowledge from these specific groups/species (i.e. mycorrhizal or pathogenic) to the fungal community as a whole from regional to continental scales in efforts to better characterize their contributions to ecosystem functioning. This has become largely possible due to technological advancements that have made extensive sampling and deeper sequencing more accessible. In this study we use Illumina high throughput sequencing to characterize the fungal community in soil and litter across a mixed temperate forest and examine factors influencing the distribution of species, specifically looking to address the influence of space.
We carried out a one-time spatially explicit sampling of a 100km^2 area, collecting soil and litter at a total of 64 sites on a 1km^2 point grid. Vegetation surveys were also conducted at the sites. Samples were analysed for soil properties and extracted DNA was amplified using general eukaryotic primers targeting the internal transcribed spacer 2 region (ITS2).
Results/Conclusions
The 64 sites covered a range of above ground diversity varying from open sites to coniferous and deciduous-dominated site with 98% cover (eg. richness 4-42spp; tree cover 10-98%). Soil and litter communities differed significantly in community composition but not in richness (litter: 2162, soil: 2163, total:2183). Litter communities were significantly correlated with tree-layer richness, moisture, pH and CN. The measured environmental variable most strongly relating to fungal community composition was horizon indicating consistent preference of some species for one horizon. Both soil and litter communities exhibited further spatial patterns after de-trending with larger spatial patterns in litter and finer spatial patterns in soil.

Plasmid community adaptation in long-term copper contaminated soil as revealed by a comparative mobilome approach

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A mobilome consists of all mobile genetic elements of the genome that is intrinsically mobile (e.g., integrated conjugative elements, plasmids and genomic islands), or is the result of identi?able horizontal gene transfer (HGT) events. Different mobilome methods (e.g., plasmidome and plasmid metagenome) separating plasmids from chromosomal DNA in environmental samples directly have been established in rumen, wastewater and sludge samples but not in soil samples. In this study, we collected two plasmid gene pools, one representing long-term $CuSO_4$-contaminated soil (around 90 years) and the other representing a corresponding non-polluted control soil in Denmark. The total plasmid DNA was acquired by a culture-independent mobilome protocol developed in our group where bacterial cells were obtained by nycodenz solution, plasmid DNA extracted with a commercial kit was digested with exonuclease to remove chromosomal and sheared DNA, and intact plasmid DNA was amplified with ?29 polymerase. The enriched plasmid DNA was then sequenced via Illumina HiSeq paired-end protocol.
The introduction of copper resulted in an accumulation of key functions resisting heavy metal ions, organic solvents and antibiotics, such as the cation efflux and ABC-type transport systems. When looking at the differences in the Bray-Curtis distances of gene functions, antibiotic and plasmid replication profiles the comparative analysis of the two types of soils reveals that an adapted bacterial plasmid community performing crucial functions has replaced the former plasmid community of the non-polluted soil. Because $CuSO_4$ was the dominant and solo contaminate, it provided the possibility to investigate the co-selection of heavy metal and antibiotic resistance. We observed a statistical significant increase abundance of antibiotics and metal and biocide resistance genes in the polluted soil mobilome suggesting that multidrugs efflux pumps confer the bacterial community with the ability to resist heavy metals and promote the antibiotic resistance as well. Also the higher abundance of transposases genes in the polluted soil might provide an evidence of more frequent mobilome genetic exchange activities in a perturbated environment.
In conclusion, this study show how plasmids and circular mobile genetic elements adapt and respond to long-term heavy metal selection pressures in the soil environment.

Dynamics of microbial communities in pre-exposed and pristine soils in response to high concentration of biochar

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The demand for green energy production is steadily increasing and, consequently, also the production of its by-products. Recycling of gasification biochar (GBC) in soil is a point of convergence between biomass conversion and by-product reuse. For such change in agricultural practice, however, the effects on the soil functioning has to be assessed prior to general implementation.
The main objective of this study was to test the differential responses between pristine soil vs. pre-exposed soil to BGC after amendment with a high dose of GBC (5% wt/wt) during three months. We hypothesized that a high dose of GBC can change the overall soil microbial community and the functional diversity, with more severe effects to be observed in pristine vs. pre-exposed soils. Furthermore, we hypothesized that GBC will not have long lasting impact on soil microbial communities and soil functions, representing a relevant candidate for linking bioenergy production and sustainable biomass cycling.
The potential effect of a high dose of GBC on soil microbial communities and their functions were tested in controlled soil microcosms. Effects on the active microbial community were measured via 16S and 18S qPCRs and via the Most Probable Number estimation of protist abundance (MPN. Functional genes were used as specific markers for measuring the variation of microbial gene expression profiles along the incubation (cDNA qPCRs of pre-exposed vs. control soils). We targeted specific genes for functions such as utilization of GBC-derived compounds, as well as utilization and tolerance providing evidence of the effect of GBC on the microbial functional response. We also measured exo-enzymatic activity related to key biogeochemical cycles to monitor any alteration in the soil catabolic potential. We explored the link between active functions and potential processes in soil, and the short versus long lasting effects of GBC on soil microbial community size and functions will be discussed.

Coping with copper: Soil active bacterial communities following 100 years of exposure

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Since the 18th century, copper derived pesticides have been massively used for wood impregnation and plague control in agriculture. However, little is known about their long-term impact on soil microbial communities and their related ecosystem services.
A former wood impregnation site in Hygum, Denmark, was intensively contaminated with copper in the early 20th century. Today, the site represents nearly 100 years of permanent local exposure and offers a remarkable opportunity to shed light on this problem. A very stable and sharp contamination gradient was discovered, ranging from normal soil copper levels to more than 4 g Cu kg^{-1}.
Our aim was to assess the structure of the potentially active soil bacterial communities along the copper gradient. For that purpose, we established an integrated approach combining metagenomics and metatranscriptomics, followed by amplicon sequencing of 16S rRNA gene transcripts.
Three areas of 16 m^2 located along the copper contamination gradient were selected, corresponding to a control (?20 mg Cu kg^{-1}), a semi-contaminated (?500 mg Cu kg^{-1}), and a hot spot area (?4 g Cu kg^{-1}), and sampled every 3 months over the course of a year.
The results indicate extreme differences in the life-style of bacteria in the three different areas, the copper being the main driving force. Increased copper concentration was associated with significant loss in bacterial richness, including key taxa involved in key biogeochemical cycling of C and N cycles. Seasonal fluctuations were higher in the hot spot compared to the other sites, indicating a lack of stability mainly due to the highly restrained and specialized microbial community.
Our data contribute with key elements for understanding evolution of soil quality after long-term exposure to recalcitrant pesticides and how this perturbation may impact soil ecosystem services and functionality.

c-di-GMP related genes are common on plasmids - a comparative analysis

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In most bacteria, the secondary messenger c-di-GMP is central for facilitating a behavioral response to different environmental clues. Specifically the transition between biofilm and motility behaviors has been linked to the intracellular lever of c-di-GMP in many different bacteria. High c-di-GMP levels typically induce biofilm phenotypes while low levels induce motile phenotypes. Here we focused mainly on two types of proteins, namely: diguanylatecyclases (DGCs) and phosphodiesterases (PDEs), which are respectively involved in synthesis and degradation of c-di-GMP. We show that genes associated with the response and turnover of c-di-GMP are much more commonly found on plasmids than hitherto imagined, and that the density of such genes seemingly are higher on these plasmids compared to chromosomes. Degenerate versions of the active sites of both DGCs and PDEs have been shown to function as sensor domains that can bind c-di-GMP. Interestingly, our comparative analysis suggests that there is a general difference in the distribution of genes that encode putative catalytic and sensor proteins between chromosomes and plasmids. As proof of concept we illustrate that a plasmid encoded DGC and PDE, predicted to be catalytic active did indeed change the host biofilm phenotype *in vitro* in various different species of Enterobacteriaceae. Our findings provide a strong link between horizontal gene transfer and the adaptation of basic bacterial behavior and illustrate that mobile genetic elements may impose specific phenotypes via a core regulatory system. We believe that these findings are best understood in light of theories on “genomic conflicts” and “selfish genes”.

Motility, stress responses and nutrients acquisition revealed by transcriptional profiling of *Burkholderia terrae* upon confrontation with a fungal host

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Confrontation of *Burkholderia terrae* BS001 cell populations with the soil fungus *Lyophyllum* sp. strain Karsten on soil–extract agar plates revealed that *B. terrae* modulates the expression of key genetic circuits in response to soil–mimicking environment and emerging fungal–hyphae. The stationary–phase sigma factor *RpoS*, and numerous genes under its control, were expressed to a large extent across treatments over the temporal regime. *B. terrae* apparently perceived the presence of the hyphae at early experimental stages (T1 – day 3; T2 – day 5), by strongly upregulating chemotaxis–related genes. At T2, a gene encoding a SET–domain protein (potential effector protein) was also upregulated. At the 'physical–contact stage' T3 (day 8), five clustered genes potentially involved in oxidative stress response and two genes encoding short–chain dehydrogenases/oxidoreductases (SDR), were highly upregulated. In contrast, genes related to general stress responses, i.e. *dnaE2* and *pqqC*, were transcriptionally downregulated. This study suggests that *B. terrae*, from a stress–induced state resulting from the soil–extract agar milieu, responds dynamically to fungal–hyphae encroaching upon it, characterized by dynamically–changing chemotaxis, metabolic signalling and oxidative stress responses.

Large Scale Spatial Analysis of Bacterial Communities in Lake Sediments, the Role of Physico-Chemical Parameters, Spatial Distance, Land Cover and Tropical Storms.

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Sediment and soils are among the most microbial diverse ecosystems on the Earth. While a variety of soils and sediments have had some of their DNA sequenced, much remains to be explored in terms of how these communities are structured, the extent of their interactions with their physical and chemical environment, and their role in ecosystem functioning. The spatial distribution of bacterial communities inhabiting sediments is highly heterogeneous at different spatial scales, but is still mostly unexplored. Some studies have suggested links between the spatial diversity of soil microbes and soil physicochemical parameters (e.g., relationship between soil pH and *Acidobacter* abundance). In this project, we hypothesize that heterogeneity of the bacterial community composition varies at the same scale level of the heterogeneity of sediment chemical properties. Here, we focused on the large scale (km) diversity. The large scale physical and chemical characteristics that we hypothesize influence microbial communities in lake sediment at the kilometer scale are land cover, climate, pH, and salinity. We tested this by examining the spatial distribution of bacteria and physical and chemical parameters in sediment of the second largest brackish lake in the world (Chilika Lake, India). Seventy-two samples (24 stations, 3 seasons-winter, rainy and summer) of sediments from Chilika Lake were analyzed by 16S rRNA gene pyrosequencing. Land cover analyses were performed using satellite images and a digital elevation model with geographic information system (GIS), and a large set of physico-chemical analyses (e.g., pH, turbidity, salinity, conductivity) were also performed on the water column over the sediment. After a hurricane passed near the lagoon in 2011, more samples were collected to see the impact of the tropical storm on the spatial distribution of bacteria in the sediment. The results of 16S rRNA gene analysis and physical and chemical parameters used with the spatial analysis demonstrated clear spatial relationships between physico-chemical parameters (salinity), land surfaces (drainage area, type of vegetation…) and extreme events, and the distribution of sediment microbial communities.

Disentangling fine soil fauna-microbial interactions in mediating key soil processes under different land-use intensity systems and climate change scenarios

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Ecosystem processes, which result in the provision of ecosystem services, rely on multitrophic interactions between functionally dissimilar soil organisms across all size classes and trophic levels. The activity and survival of many soil organisms depend on the presence of other soil biota acting at different spatial scales with complementary functions. Their interactions often increase processes rates.
Experimental research on the effects of change or loss of soil biodiversity as a consequence of external factors, such as climate change and land management, may provide important indications of the impact on soil species, on ecological processes and on the provision of associated soil ecosystem services.
We investigated how different rain regimes affected the soil community structure and activity of an agro forest system with two different land managements (sustainable and conventional production) in the South of Portugal. A mesocosms experiment using Terrestrial Model Ecosystems was performed during six months. The resistance (1st to 3rd month) and the resilience (4th to 6th month) of the soil community from both sites were tested by the application of five different rain regimes, from drought to flood events.
The sustainable site, showed in general, a profile of metabolic activity lower than the conventional site. Both sites exhibited shifts in the microbial community as a result of the rain treatments, yet, these were more pronounced in the intensive rain regimes when causing flood conditions. The higher process rates found in the conventional site suggest that its soil community has a dynamic capacity to respond to climatic stresses, most probably as a result of the previous and recurrent harsh conditions caused by the corresponding land management. These higher rates also indicate an opportunistic soil community with high efficiency in the use of resources for metabolic needs. When tested, the resilience for both agro forest sites, i.e., within the same rain regimes, the sustainable site showed a slightly better recovering despite the lower rates in the assessed processes comparing to the conventional site. This suggest a more balanced soil community that allowed both moderate shifts and activity rates in the soil community over the experiment, indicating a “buffer soil community effect” that resisted better to the climatic period of stress.

Using CRISPRs to learn about virus-host interactions in the environment

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Interactions between the members of a microbial community can enhance their adaptation to environment conditions and changes. Among the many interactions that take place in an ecosystem, the one involving phages/viruses and their hosts has been seen to play a major role in microbial diversity and population dynamics. While viruses and phages can mediate the transfer of genetic material between microorganisms (transduction), the relationship between them and their hosts in natural environments is poorly understood. Thus, measuring the impact that transduction might have on microbial communities is a difficult. We propose using clustered interspaced short palindromic repeats (CRISPRs) as a tool to explore viral dynamics and viral-host interactions in the environment. By retaining viral sequences from past infections, CRISPRs provide information on viral dynamics, such as host ranges and viral diversity. Moreover, CRISPRs could help link viruses and phages with their microbial hosts.
Viruses in the cryosphere have been reported to be abundant and suggested to be highly active and with broad host ranges. These characteristics could explain why viral transduction might be a key driver of adaptation in these kinds of environments. We, therefore, used CRISPRs to explore the possible relationship between temperature and viral dynamics using public metagenomes. Infection networks were created where viruses were connected to the microbial cells they infect using CRISPRs issued from arctic ice and soil metagenomic data. Finally, we searched for transduction events in these cold environments to evaluate the adaptive significance of transduction in the cryosphere.

Phylogenetic and taxonomic diversity of glycoside hydrolase family 5 and 48-cellulase genes in agricultural soil

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Cellulose degradation strongly influences carbon sequestration rates of soils and thus contributes to soil quality and the formation of organic matter. Cellulase genes show a great diversity and are harboured by many different microbes. This leads to the question whether the ability to degrade cellulose developed mainly by co-evolution or if horizontal gene transfer was the major driver. The phylogenetic diversity and taxonomic distribution of cellulases in bacteria has been analysed so far using protein- and whole genome sequences from public databases, but it is not known if the observed patterns reflect the real in situ-situation. Here, we compared the phylogenetic diversity and taxonomic distribution of potential cellulases of glycoside hydrolase families (GHF) 5 and 48 in an agricultural soil using amplicon sequencing.
To this end, the metagenome of an agricultural soil was sequenced (de Vries, Schöler et al., 2015) and annotated cellulases were used as a basis for the development of specific primers. Primer products were sequenced and potential cellulases were identified by pairwise alignment and Markov Clustering, together with characterized cellulase sequences derived from the CAZy database (http://www.cazy.org). Taxonomic annotation of the annotated cellulases was performed by blasting the translated sequences against the NCBI protein database. The evolutionary rate of the GHFs was calculated using the DAFGA-analysis method (Kim and Liesack, 2014).
The phylogenetic analysis of potential GHF 5 and 48-cellulase genes in amplicon- versus metagenomic sequencing data shows a comparable taxonomic annotation. We will further show a comparison of the evolutionary rate of cellulase genes within and between these families. The non-monophyletic distribution of cellulase genes for each family indicates that horizontal gene transfer has likely played a significant role during evolution of these genes in agricultural soil.
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Are the most abundant bacteria real key players in forest soil processes? A multi-omics approach.

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Coniferous forests represent a large C sink in the northern hemisphere and are thus of global importance. The main objective of the present study was to disclose the metabolic capabilities of abundant bacterial strains from a *Picea abies* forest soil in the Bohemian Forest Natural Park, Czech Republic and depict their importance in ecosystem processes. Isolation, physiological characterization and whole genome sequencing of abundant bacterial taxa in the soil and litter horizons was performed. Low nutrient medium (pH 4.5) was used to isolate the maximum number of bacterial strains with high abundance in the acidic soil. After identification, enzymatic activity and C-source arrays were used for physiological characterization of isolates as well as whole-genome sequencing from isolates and single cells. Sequencing results revealed that Proteobacteria, Acidobacteria and Actinobacteria were dominant in both the litter and soil, comprising 85-90% of all sequences from DNA and RNA. In total, 299 bacteria were isolated of which two (*Bradyrhizobium* and *Methylocapsa*) rank among the five most abundant OTUs in the soil. Proteobacteria, Actinobacteria and Acidobacteria were the predominant phyla among the isolated bacteria (65%, 17% and 13%, respectively). The physiological characterization of environmental strains showed that soil bacteria are relatively versatile in their ability to use soil-derived carbon sources. Annotated gene content of selected strains indicated presence of CAZy, ammonification and denitrification genes, suggesting these bacteria play a role in important ecosystem processes such as soil carbon and nitrogen turnover. Moreover, individual bacterial genomes were used to identify the genes and transcripts of studied taxa in the soil metagenome and metatranscriptome.

Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in Coniferous forest soil

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Organic matter decomposition plays an imperative role in the carbon cycle, exclusively in the global widespread coniferous forests. Cellulose degradation is very important in this respect because cellulose is one of most abundant polysaccharide in the plant litter. Potential and active β-glucosidase producing microbial communities were studied in top soil of Picea abies forest in two contrasting seasons. These two seasons were late summer at the peak of plant photosynthetic activity and late winter an extended period with no photosynthetic input. In order to analyse the succession of functional community by PCR- amplicon sequencing approach, two set of PCR primers were designed for specific amplification of fungal ?-glucosidase from CAZy glycoside hydrolase gene families GH1 and GH3 and also two set of published primers were used for specific amplification of bacterial β-glucosidase for GH1 and GH3 families. RNA-derived population shows parallel microbial population as DNA-derived population, but ominously diverge in the composition of microbial taxa and many highly active taxa shows low abundance or even absence in the DNA pool. In fungi, Ascomycetes and basidiomycetes and in bacteria, firmicutes, actinomycets, proteobacteria, acidobacteria and deinococcus-thermus being major reservoirs of β-glucosidase genes which shows that cellulose degradation is mediated by highly diverse microbial populations. Variations in periodic functioning is the combination of adequate alterations in potential diversity and intense changes in transcriptional profile, specifically in fungal β-glucosidase gene diversity. In fungi, these variances were highly significant in litter horizon and differences were more significant in soil for bacteria. Results indicate that both bacteria and fungi are playing important role in β-glucosidase enzyme production and also in the cellulose decomposition. The low abundant species have important contribution to cellulose degradation processes and plant photosynthetic production was likely the key driver of changes in the functional diversity in the studied ecosystem seasons.

Autogenic succession in the soil microbial community

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In a world in which natural ecosystems are under increasing pressure of environmental change, understanding how communities recover is crucial to predicting their ability to function in the future. In microbial ecology, while the relationship between community composition and the environment has been extensively studied, the biotic—or autogenic—factors driving community dynamics and the potential for internal feedbacks have not been rigorously explored. Here, we subjected highly controlled soil microcosms to a heat disturbance and followed the potentially active microbial community composition over 50 days of recovery using 16S rRNA amplicon sequencing. As expected, the disturbance acted as a selective pressure, increasing the system's deterministic turnover immediately after the disturbance. Surprisingly, however, this determinism was even stronger on the fourth day of recovery, and then gradually decreased throughout the rest of the experiment, suggesting that the disturbance trigged successional dynamics. While Proteobacteria were negatively affected by the disturbance, we found that Acidobacteria, Chloroflexi and Clostridia also decreased in abundance within the period studied, but not immediately after the disturbance. The effects of the disturbance were prolonged, and clustered in time. We thus identified four stages in the microbial community's response: a primary response (1 day after disturbance) in which surviving copiotrophs increased in abundance, a secondary response (4 days after disturbance), in which some surviving taxa and copiotrophs were displaced and rare taxa became abundant, a tertiary response phase (10-29 days), during which community dynamics slowed down, and a stability phase (after 29 days), during which taxon richness, but not community composition, returned to its original value. The absence of environmental variation following disturbance allowed us to attribute community dynamics to autogenic factors. The changes observed during this period were very large, affecting up to 30% of the community within a sampling interval. Importantly, these dynamics led to the displacement of taxa which were originally unaffected by the disturbance itself, as well as to the dominance of initially rare taxa. Thus, the community's response to disturbance is far from linear: the recovered community's composition is dependent not only on the sensitivity of its members, but on the successional dynamics resulting from the newly available niche space.

Effects of land use on soil ciliate diversity

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Land use intensification is one of the most eroding processes for biodiversity with likely feedbacks on ecosystem functioning. Is known to have negative effects on the diversity of above and belowground communities. As microorganisms play key roles in nutrient cycling and ecosystem functioning of soil, it is well recognised that more knowledge on their specific effects is needed. However, most studies focus on the spatial variation in the diversity and composition of soil bacterial communities, ignoring the soil eukaryotic microbes and thus, making it difficult to assess overall land use effects. Ciliates as one of the most successful group of protists on Earth, colonize and inhabite virtually all environments, making them a key functional group within the soil microbial loop. Changes in soil ciliate diversity have been suggested as bioindicator of environmental stress. Despite this, little is known about overall diversity of soil ciliates, and effects of land use and soil physicochemical factors on soil ciliate diversity.
In this study we investigate soil ciliate diversity in soils sampled across European geographical locations and climatic zones, covering different vegetation types and land uses. Both quantitative and qualitative methods to investigate the influence of land use types on protist communities were applied. An 18S amplicon approach based on general 18S rRNA gene primers and an additional specific primer for ciliated protist group were used in a high-throughput sequencing (HTS) approach. Our results demonstrate that the diversity differs significantly between geographical locations. Likewise, land use and soil physicochemical factors also influenced the overall ciliate community. Soil ciliate communities exhibited compositional shifts that followed changes in land use and soil management. Hence, agricultural management can have large effects on this protist community. These findings contribute to future research for bio-indicators of soil quality and sustainable land use and upon further development of the metabarcoding approach for the different soil ecologically relevant protist taxa like amoebae and cercozoans, this technique is a promising indicator of soil protist diversity.

Understanding soil quality and biodiversity

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Soils holds a primary strategic importance by providing support for plants and organisms and therefore for the life of all human beings. The soil system is dynamic, highly heterogeneous and extremely complex. In this aspect, soils are by far one of the richest ecosystems. This biodiversity represents a vital component to the contribution of soil quality. Fertile soil is essential for human survival. However, threats such as pollution, intensive use of land and climate change are placing increasing stresses on the ability of soil to sustain its important role in the planet's survival.
Trainbiodiverse project, a multi-disciplinary and international network of European research institutions and independent private sector working in collaboration, aims to understand the links between soil biodiversity and soil quality by developing new methodologies and analysis toolbox and provide critical information to enhance knowledge in soil quality, ecosystem services and economic significance of soils.
Water and Soil Remediation Company (WSR), an Italian leading company for Environmental solutions participates as a full Partner within the Trainbiodiverse project. WSR believes that technological development and scientific research significantly contributes to the improvement of the offered services and also to the Client satisfaction.
Thanks to this challenging opportunity, WSR by hosting and training an Experienced Researcher has contributed to the research and identification of some parameters that influence soil quality. In order to achieve this objective, several soil samples from polluted, stressed or non-polluted sites have been analyzed and a soil database has been created. All the information gathered will help to individuate the parameters that influence soil quality.
On the other hand, the subsequent dissemination and training activities organized also outside the network have contributed not only to the improvement of the scientific knowledge in soil biodiversity and ecosystem services but also to raise awareness in the importance of soil health. This will be surely a precious occasion to grow new ideas and projects for the soil biodiversity and quality preservation.

Proteolytic soil communities and protease activity in rhizosphere of maize plants with different Nitrogen Uitilizing Efficiencies(NUE)

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Nitrogen Utilizing efficiencies(NUE)of plants is an inherent plant property regulated by complex genetic and metabolic factors. NUE is closely related to rhizosphere activities and it has been well investigated in many agricultural crops. A broader understanding on proteolytic microbial populations can help us in identifying their roles linked to plant NUE. This study investigated the interplay of plant NUE with N mineralization brought about by proteolysis with special focus on bacterial extracellular soil proteases.
We hypothesized that plants with different NUE could select different proteolytic microbial populations in the rhizosphere, characterized by different levels of proteolytic activity. We studied changes in the biochemical activity and microbial composition structure in the rhizosphere of the inbred maize (*Zea mays* L.) lines Lo5 and T250 characterized by high and low NUE using rhizobox experiments. Plants were regularly monitored for the inorganic N (NH4+-N and NO3--N) concentration in the rhizosphere by ion selective electrodes (ISE) and on N depletion samples were collected from rhizosphere and bulk soils. Microbial biomass was estimated as a measure of ATP, and protease activity was assayed as N-benzoyl-L-argininamide and caseinate hydrolyzing activities. Two bacterial genes coding for alkaline proteases (apr) and neutral protease (npr) were studied. Bacterial gene abundance was measured using qPCR. Morevover to study diversity of these genes, amplicons were sequenced using the Illumina method.
Plant L05 showed a quicker uptake of inorganic N, higher activity of proteases and a higher ATP content. Plant L05 also, showed a significant difference in the rhizospheric and bulk microbial biomass, whereas plant T250 showed no significant differences in the microbial biomass of rhizosphere and bulk soil.Furthermore, abundance of genes apr and npr and their diversities, as indicated by qPCR and sequencing results, respectively, were favored by the higher NUE of L05 maize line. These results shows that L05 plants with higher NUE, harbour a diverse selection of soil microbial communities involved in proteolysis. Most apr OTUs were from uncultured bacterias and Bacillus sp., most of the npr OTUs were from members of *Pseudomonas* sp.
This study provides useful information related to soil proteolytic microbial populations that can be helpful to improve the NUE of agronomically important plants, even at crop scale.

Bacterial growth responses to drying/rewetting and freezing/thawing – a tale of two patterns

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Drying /rewetting and freezing/thawing are examples of transition events in soil, where a situation of no or little growth is rapidly turned into a situation with good conditions for growth with ample supply of substrate. Two patterns of bacterial growth development have been observed upon rewetting dry soil. In the type 1 pattern bacterial growth starts immediately upon rewetting and increases linearly with time up to values similar to or slightly higher than in constantly moist soil. This pattern has the highest respiration immediately after rewetting, decreasing exponentially with time. In the type 2 response the initial bacterial growth is very low after rewetting, and starts increasing exponentially only after a pronounced lag period. Growth eventually reached values much higher than in constantly moist soil. The exponential growth often coincides with a secondary increase in respiration.
Both drying conditions and pre-treatments of soil will affect the response type that will result after rewetting. Duration of drying and storage of soil before drying/rewetting will result in a change from a type 1 response to a type 2 response, while milder drying (to higher moisture content than air dried soil) and repeated drying/rewetting cycles will result in a change from a type 2 response to a type 1 response. Temperature appears not to change the response type, but will affect the rate of the transition. Comparison with freezing/thawing responses will be made, as well as with other transition events, like sudden increases in substrate concentrations. All these transition events appear to induce an initial “decoupling” between growth and respiration, possibly altering carbon use efficiency.

Zooming in on the Functional Heterogeneity of Ammonia Oxidizing Archaea in Arctic Soils

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Since their discovery about 10 years ago, the distribution and abundance of ammonia-oxidising archaea (AOA) has been compared and contrasted in numerous studies to that of their bacterial counterparts (AOB). However, functional heterogeneity among different lineages of AOA was difficult to assess and compare among studies, due to inconsistent phylogeny of the amoA marker gene used in the surveys. With a new analysis pipeline that takes into account problems inherent to commonly used protein marker analyses, we have now infered a stable phylogeny based on all ~45.000 available archaeal amoA gene sequences from public databases. It can be used as a robust taxonomic and phylogenetic framework to dissect specific AOA lineages and link their occurrence to environmental parameters.
In order to analyse the effect of climate change on AOA populations, cryoturbated arctic soil from the upper organic layer and from subducted organic material of deeper horizons was incubated under different temperature and moisture conditions (within the international project CryoCarb). Differing and sometimes contrasting dynamics of AOA subpopulations were observed in this experiment indicating that AOA need to be analysed at higher resolution than usually done, if we want to characterize the reaction of AOA populations to changing environmental conditions - an observation that holds probably also true for other functional microbial guilds in terrestrial environments.

Plasmid-mediated adaptation of soil bacteria to pollutants

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Plasmids often carry genes conferring various fitness traits. We hypothesize that under non-selective conditions in soils only a minor proportion of cells carry plasmids. However, plasmid-containing cells might become important when soil bacteria are confronted with pollutants as plasmids ensure a rapid adaption to changing environmental conditions. Pollutants can either be degraded or cell toxicity is reduced by various mechanisms encoded by plasmids. Thus in the presence of pollutants typically an increased relative abundance of plasmid-containing cells is observed resulting from the proliferation of plasmid-containing cells and horizontal gene transfer processes. The research presented focused on plasmid-mediated adaptation of soil bacteria to antibiotics and to pesticides.

The use of antibiotics in animal husbandries is assumed to be an important factor contributing to the environmental pollution with antibiotic resistance genes (ARGs). Spreading organic fertilizers such as manure and digestates onto agricultural soils was shown in several studies to introduce not only nutrients but also bacteria carrying transferable antibiotic resistance genes and antibiotics. The fate and effects of antibiotics on soil bacterial communities depend on the physico-chemical properties of the antibiotics and on the soil properties. Cultivation-independent methods such as exogenous capturing of plasmids and quantification of plasmid-specific sequences in total community DNA were used to elucidate the types of conjugative or mobilizable plasmids involved in spreading ARGs among soil or plant-associated bacteria. We most frequently captured LowGC, IncP-1 and IncQ plasmids directly from feces, manure, digestates and soils. Sequencing data provided insights into their evolution and the co-selection of multiple ARGs on the same elements. Competition studies demonstrated that the metabolic burden of a LowGC plasmid in Acinetobacter baylyi turns into a fitness advantage in soils treated with antibiotic-containing manure.

The application of pesticide-containing water to material from on-farm biopurification systems also resulted in an increased relative abundance of IncP-1 plasmids under microcosm and field conditions and 454 amplicon sequencing of an IncP-1- specific sequence revealed a comparable dynamic of IncP-1beta and IncP-1epsilon groups Presently, plasmids captured directly into *Pseudomonas* putida are investigated for the presence of degradative genes.

A case-study for traits-based theory and prediction in microbial ecology: colonisation of sterilised soils across a pH gradient

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We compared the influence of community and environmental conditions for the functioning (fungal and bacterial growth and respiration) and trait distribution (bacterial pH-tolerance) of soil microorganisms across a pH gradient. A reciprocal inoculation experiment, including pHs 4.1, 5.2, 6.7, and 8.3, was used. Sterilised soil microcosms with added plant material were inoculated with unsterilized soil and monitored during two months. Respiration was dominated by bacteria at high and by fungi at low pHs. The bacterial pH-tolerance of all inoculated communities (initial trait distribution) converged with the pH of the soil (environment). There were also differences between community inocula, resulting in suboptimal pH-tolerance when the inoculum pH did not correspond to soil pH; low pH inoculum communities had lower than optimal pH-tolerance in high pH soils and vice versa. Bacterial communities with traits misaligned to their environment had impaired functioning (growth in all soils and respiration in high pH soils). The inoculum community effect on bacterial pH tolerance and functioning could be detected within one week and remained for two months. Fungal communities emanating from low pH inocula resulted in higher fungal growth and biomass (in all soils) and respiration (in low pH soils). Variation in fungal pH-tolerance traits thus did not influence their performance, in contrast with bacteria. Instead the fungal inoculum size from low pH soils appeared to explain these results. Consequently, respiration was characterised by the alignment of the bacterial trait distribution to the environment for high pH soils, and by a faster growing fungal inoculum in low pH soils.

Metabolic and trophic interactions modulate methane production by arctic peat microbiota in response to warming

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Arctic permafrost soils store large amounts of soil organic carbon (SOC) that could be released into the atmosphere as methane (CH_4) in a future warmer climate. How warming affects the complex microbial network decomposing SOC is not understood. We studied CH_4 production of Arctic peat soil microbiota in anoxic microcosms over a temperature gradient from 1 to 30 °C, combining metatranscriptomic, metagenomic, and targeted metabolic profiling. The CH_4 production rate at 4 °C was 25% of that at 25 °C and increased rapidly with temperature, driven by fast adaptations of microbial community structure, metabolic network of SOC decomposition, and trophic interactions. Below 7 °C, syntrophic propionate oxidation was the rate-limiting step for CH_4 production; above this threshold temperature, polysaccharide hydrolysis became rate limiting. This was associated with a shift within the functional guild for syntrophic propionate oxidation, with Firmicutes being replaced by Bacteroidetes. Correspondingly, there was a shift from the formate- and H_2-utilizing Methanobacteriales to Methanomicrobiales, and from the acetotrophic Methanosarcinaceae to Methanosaetaceae. Methanogenesis from methylamines, probably stemming from degradation of bacterial cells, became more important with increasing temperature. This corresponded with an increased relative abundance of predatory protists of the phylum Cercozoa. We concluded that Arctic peat microbiota responds rapidly to increased temperatures by modulating metabolic and trophic interactions such that CH_4 is always highly produced: the microbial community adapts through taxonomic shifts, and cascade effects of substrate availability cause replacement of functional guilds and functional changes within taxa.

Impact of climate change on carbon cycling and soil microorganisms in an arable ecosystem

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Carbon cycling in terrestrial ecosystems provides a feedback mechanism to climate change by releasing or sequestering additional atmospheric CO_2. However, the role of soil microorganisms as key players in this feedback mechanism is still unclear. The objective of this study was to link microbial abundance and activity to SOC turnover under conditions of climate change. Specifically, we hypothesized that 1) a modified temperature and precipitation regime will change microbial abundance and activity, which will be accompanied by a season specific change in SOC turnover rates. The Hohenheim Climate Change (HoCC) experiment was established in summer 2008 to manipulate soil temperature and precipitation on an arable field. Soil temperature is increased by 2.5°C in 4 cm and is combined in a factorial design with the following precipitation manipulation treatments: a) ambient, b) precipitation amount decreased by 25% during summer, c) reduced precipitation frequency by 50% during summer, d) combination of b and c. Soil samples (0-5 cm, 5-15 cm and 15-30 cm depth) were taken at 15 dates from 2009 to 2013. Data of aboveground biomass, soil organic carbon, soil microbial biomass, ergosterol content, hydrolytic enzyme activities and CO_2 fluxes will be presented. Effects of elevated soil temperature on microbial biomass and CO_2 fluxes were related to moisture conditions during the different seasons of the year in 2009 but not in the following years. In the years 2010-2012, warming increased soil respiration across the seasons. Effects of reduced summer precipitation on soil respiration could be explained by the yearly weather conditions as a boundary condition for the response of soil respiration to climate change; specifically, we found a linear relationship between the precipitation amount in May, i.e. moisture availability at the start of the precipitation manipulation period, and the reduction in CO_2 emission by reduced summer precipitation. First evaluation of ergosterol contents and enzyme activities from 2009 to 2012 indicate that soil warming generally stimulate fungal biomass and microbial activity depending on the time of sampling. Overall, soil warming was the most effective factor in the HoCC experiment showing a continuous enhancement of microbial activity and CO_2 emission. The presentation will give insight into the complex interactions between climate change, soil moisture and soil microorganisms as key players of carbon cycling.

Ectomycorrhizal and non-symbiotic fungi respond differentially to climatic parameters: what is the link with host susceptibility to climate change?

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The impacts of climate change on forest biodiversity have been well documented for plants and vertebrates, revealing a high sensitivity of certain plant species to temperature and rainfall. But climatic and other environmental factors that maintain and structure microorganism diversity are still unclear, in particular for the different ecological groups of fungi. Unlike free-living saprobes, ectomycorrhizal fungi are intimately associated with the roots of forest trees, where they promote tree growth through nutrient and water uptake. This symbiotic trait suggests that these fungi could show climate sensitivity close to that of their hosts, and potentially stronger than saprotrophic fungi. To test this hypothesis, this present study aims at identifying climatic and edaphic parameters which impact fungal diversity by focusing on ectomycorrhizal fungi associated with Fagaceae (aak and beech). We set up a long-term monitoring experimental network for assessing the impacts of forest management and climate on soil fertility and soil biodiversity. Eleven forest sites (*Fagus sylvatica* and *Quercus petraea*), 2 ha each, have been selected from the "MOS network" (for "Soil Organic Matters"), because they represented a large regional rainfall gradient. Using Illumina MiSeq technology, richness and structure of the different ecological categories of fungi have been studied in relationship with edaphic and climatic factors. Richness was mainly driven by pH and carbon and calcium concentrations for both non-symbiotic and symbiotic fungi. As previously reported, we confirmed that free-living soil fungi were primarily impacted by the soil pH. Interestingly, nonmetric multidimensional scaling, revealed that, unlike saprotrophic fungi, ectomycorrhizal fungi assemblages are firstly driven by tree hosts species and by mean annual precipitations, especially under the beech stands. The ecological responses of ectomycorrhizal communities could be directly linked to the climatic sensitivity of their tree hosts, in particular Fagus sylvatica. In the light of global change factors, these results confirm the need to develop joint ecological analyzes of tree species and their ectomycorrhizal partners. Finally, we will present ongoing research on this national-scale experimental network (MOS network) that will extend our understanding of forest ecology and management, partly by focusing the coupled effects of climate and biomass and organic matter removal.

Vulnerability of permafrost carbon to climate change

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Arctic permafrost soils contain more than 1300 Pg of organic carbon (C) about half of the global soil C and twice as much as is currently contained in the atmosphere. They are, therefore, important components in the global C cycle, having acted as C sinks since the beginning of the Holocene. Substantial amount of this C is stored in buried pockets of organic matter (OM) by the process of cryoturbation. Cryoturbated soils contain more than one third of the arctic soil C. However, the information about their decomposability is scares. We here present results from the project that aimed at identifying the role of microbial functioning and decomposition of OM in cryoturbated soils from Siberia and at accessing the potential vulnerability of this OM in future climate. Our main findings were: 1. Temperature is not main control for decomposition of cryoturbated OM. Cryoturbated OM with comparable C content exhibited continuously lower respiration rates and methane production, demonstrating that factors other than temperature must control OM decomposition. 2.The microbial community is not able to decompose the organic matter in cryoturbated pockets. The microbial biomass of the buried material was very low and microbial community distinctly different from topsoil and more similar to subsoil microbial communities. It thus seems that there was a mismatch between microbial community and organic matter quality that added to the retarded decomposition of cryoturbated OM. 3.In cryoturbated OM nitrogen availability is reduced and N cycling decelerated. In several incubation experiments including two SOM priming experiments with labelled glucose, cellulose, amino acids and protein we demonstrated different nutrient limitations of the permafrost microbial communities. While no priming was observed in topsoil, in cryoturbated material, however, the N-containing substrates led to a significant priming effect, indicating a strong N limitation of the microbial community in this soil. In summary, we are able to demonstrate that, in addition to unfavorable environmental conditions, decomposition processes in cryoturbated arctic soils are retarded by a combination of changes in microbial community composition reduced nitrogen availability and decelerated nitrogen cycling. The potential decomposability of organic matter in cryoturbated permafrost soils will be discussed.

Metagenomic insights into microbes living in the cold, extreme polar desert soils of Eastern Antarctica

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The Windmill Islands region of Eastern Antarctica is an extreme polar desert area comprised of a range of landform types, from relatively well connected catenas to patchy, patterned ground landforms, and microbes often form the major component of biomass. Remote sites within the Windmill Islands are described as pristine, and devoid of plant or animal life. Soils also range in physical properties, but are generally characterized as mineral soils, very low in water and nutrient poor. While the microbial diversity in some specific regions of the Antarctic is well described, very little information is available on the bacterial or fungal diversity present in the Windmill Islands, apart from the well-studied Casey Station. Therefore, we investigated the microbial community diversity and potential functioning of soil microbes in this region. We revealed a potential biodiversity hotspot to exist, as soils from Mitchell Peninsula and Robinson Ridge were comprised of a unique microbial taxonomic structure, with an unusually high abundance of Candidate Division bacteria WPS-2 and AD3 and Chloroflexi. With the utilization of differential coverage binning of multiple genomes we then re-constructed 18 near complete genomes from Robinson Ridge soils, including for the first time, genomes for both Candidate Division WPS-2 and AD3. The majority of genomes recovered contained a novel cluster of RuBisCO genes, which were indicative of carbon fixation. Yet, very little genetic evidence for photosynthesis exists in these soils. Discussed will be insights into the recovered genomes, with a focus on the new bacterial Phyla and primary production strategies. Overall, the community appeared to have developed novel mechanisms for survival, including a predominantly chemolithotrophic lifestyle in this extremely cold, dry, and barren landscape.

Plant invasion (garlic mustard; *Alliaria petiolata*) alters fungal community composition, increases fungal diversity, and shifts dominant fungal trophic strategy

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The non-mycorrhizal herb garlic mustard (*Alliaria petiolata*) produces glucosinolates that reduce plant diversity by inhibiting mycorrhizal fungal colonization. The plant is native to Europe and Asia and was introduced into North America in the late 1800's, where it is now a destructive invasive species. Invasion can suppress root mycorrhizal communities, but little is known about how invasion impacts the entire soil fungal community, where saprotrophs, plant pathogens, parasites, and mycorrhizal hyphae reside. Our objective was to characterize the fungal community structure of uninvaded an invaded soils from replicate plots established in six deciduous forest understories in the Northeastern USA. We used Illumina metabarcoding (ITS2), phospholipid fatty acid analysis, and characterized edaphic soil properties. Fungal genera were also annotated by trophic strategy (saprotroph, symbiotroph, & biotroph) and mycorrhizal type (ectomycorrhizal; EcM or arbuscular mycorrhizal; AMF). In terms of soil properties, invasion was associated with lower organic carbon content, elevated soil pH, and higher soil nitrate concentrations, but no difference in fungal or bacterial phospholipid fatty acid abundances. The fungal community in invaded organic horizon and mineral soil was more diverse, compositionally distinct, and homogenous relative to uninvaded communities. Uninvaded soil was also dominated by EcM fungi, while invaded soil was dominated by saprotrophic fungi. The ratio of EcM to non-EcM was positively correlated with soil C:N ratios and this correlation was significantly impacted by invasion. The transition from biotrophic to heterotrophic carbon metabolism dominance may explain this correlation, as the lower C:N ratios in association with invasion were due to reduced organic carbon, not higher nitrogen. Finally, saprotrophic and biotropic (including plant pathogens, mycoparasites, and nematophagous fungi) abundances and richness were higher in association with invasion, and the richness of these two groups was positively correlated with the density of garlic mustard at a plot. Shifts in saprotrophic fungi could affect decomposition while biotrophic fungi may further suppress native plants suffering from dismantled mycorrhizae. In conclusion, invasion was linked with severely transformed soil fungal communities, comprised of fewer ECM and enriched with saprotrophs and biotrophs that may distress native above-belowground interactions and alter soil processes.

Maturation of the Field of Microbial Forensics

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The field of microbial forensics was developed to analyze evidence associated with biological crimes in which microbes or their toxins are used as weapons with attribution as the ultimate goal. Attribution of microbial evidence is to determine an associated source and perpetrator or group of individuals to the highest degree possible. Microbial forensics combines the practices of epidemiology with the characterization of microbial and microbial-related evidence to assist in determining the specific source of the sample, as individualizing as possible, and/or the methods, means, processes and locations involved to determine the identity of the perpetrator(s) of an attack. There is a wide variety of microbial species or strains that could serve as possible biothreats (human, plant, and animal pathogens). Biological signatures for biological threat agent identification and non-biological signatures, such as those that infer the culture method used, manufacturing processes, time of production, and methods of dissemination can be quite informative for developing investigative leads. Culture is still considered the gold standard for pathogen detection. However, culturing cannot provide sufficient resolution, and because there can be a substantial lag time due to growth requirements of the microorganism, it may not be efficient for response when the safety of individuals is a concern. Moreover, about 99% of microorganisms cannot be cultured by current methods; therefore culturing is not a reliable method for fastidious and possibly novel/uncharacterized microorganisms. In addition, the microbes may have been exposed to environmental insults and no longer be viable. So even if the microorganism was one of the few that could be cultured, no information would be obtained if it were nonviable. Molecular biology techniques, such as real-time PCR and massively parallel sequencing, may be better alternatives to meet the challenges of characterization and attribution. New genomic technologies and data, inclusive databases with expanded reference genomes, extensive endemic data, and validated methods all contribute to the proper interpretation of results in a microbial forensic investigation. High quality and confidence of results are essential since microbial forensic interpretations can have a large impact on society, regarding safety, political policy, and economics.

Predicting the origin of soil evidence: high throughput eukaryote sequencing and MIR spectroscopy applied to a crime scene scenario

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Soil can serve as powerful contact trace evidence in forensic casework, because it is highly individualistic and can be characterised using a number of techniques. Complex soil matrixes can support a vast number of organisms that can provide a site-specific signal for use in forensic soil discrimination. Current DNA fingerprinting techniques rely on variations in fragment length to distinguish between soil profiles, and focus solely on the microbial community. However, the recent development of high throughput sequencing (HTS) offers the potential to provide a more detailed picture of the soil community than traditional methods by allowing access to the non-culturable microorganisms, identify specific bacteria, fungi, and plants within soil as biological markers. In this study, 18S rRNA profiles of six samples recovered from a suspect's belongings in a mock-case study were compared to those collected from seven reference locations across South Australia to demonstrate the application of HTS to forensic soil analysis. Our results demonstrate the utility of non-microbial DNA to discriminate between different sites, and link a suspect to a particular location. In addition, we show that the discriminatory power of HTS is comparable to that obtained by mid infrared spectrometry (MIR) analysis, a method currently accepted in court. Through design of a mock case scenario, we highlight the considerations and potential limitations of this method in forensic casework.

Microbial Soil Community Analyses for Forensic Science: Application to a Blind Test.

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Crime-fighting is a significant concern for citizens of the European Union. Soil complexity, heterogeneity and transferability make it valuable in forensic investigations to help obtain clues as to the origin of an unknown sample, or to match samples from a suspect with a crime scene. In a few countries soil analysis is used in legal issues from site verification to body decomposition. However, up to date the application or use of soil information in criminal investigations has been limited. Comparing bacterial communities in soil samples could be a useful tool for forensic science. To evaluate the relevance of this approach, a blind test was performed to determine the origin of two unknown samples (one from the crime scene and the other from an alibi site) compared to three reference samples (soil samples from the crime scene and the alibi scene and from the farm where the suspect lived). Three biological methods were selected (Ribosomal Intergenic Space Analysis, phylogenetic microarrays and 16S rRNA gene sequencing) to evaluate the discriminating power of soil bacterial communities. Results for each method will be presented as well as the identity of the unknown and reference samples. This blind test was carried out in several labs (as part of an inter-laboratory collaborative exercise); an overview of the results will be presented.

Log decay of *Fagus sylvatica* in temperate forests and the significance of lignin modifying enzymes for the degradation process

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Deadwood is an important component in forest ecosystems and fulfills a broad set of functions like providing habitat, contribution to the nutrient cycle, to the carbon and water storage. The process of degradation of dead wood is a complex interplay of biotic and abiotic variables. Especially the process of lignin degradation is poorly understood. Filamentous fungi equipped with lignin modifying enzymes (LMEs) including laccase, manganese and manganese-independent peroxidases are the key players in the bioconversion of lignin.
We investigated the process of dead wood degradation for Fagus sylvatica, Picea abies and Pinus sylvestris coarse woody debris (CWD) across different regions and forest types in Germany. Overall 190 slightly, moderately and strongly decayed logs were selected. In dependence of the log length, 3 to 7 samples were taken from each log resulting in a total number of 739 samples.
To describe the process of degradation and to reveal the dependency of LMEs on different variables, we analyzed these samples, in addition to LME activities, for pH, water content, wood density, total lignin, organic extractives, water-soluble lignin fragments, nutrients (Al, Ca, Cu, Fe, K, Mg, Mn, N, Zn) and fungal species richness (ARISA). Moreover, sporocarps of fruiting fungi (basidiomycetes, ascomycetes) were recorded over two years.
Here we will focus on results from F. sylvatica CWD, since LMEs were significantly higher in *F. sylcatica* than in the two coniferous species. In 91% of all *F. sylvatica* samples, we detected LMEs, which fits well to the recorded presence of white-rot fungi. The pattern of LMEs was highly variable, pointed by a mean variation coefficient of 125% within each log. Logistic regressions and correlations between enzyme activities and the variables revealed that the amount of water-soluble lignin fragments, water content and fungal community structure are important determinants. As result of the fungal decay activities, most wood constituents were degraded and/or released in soluble form. This was particularly pronounced in the case of lignin, organic extractives and most nutrients, the amounts of which decreased in relation to the wood volume with ongoing decay.
Based on this large field study, we conclude that LMEs in CWD are highly relevant for lignin degradation. The variable pattern of their secretion is the result of a complex array of wood parameters and the fungal community structure, which has only partly be resolved so far.

Divergent effects of pyrochar and hydrochar on greenhouse gas emissions and microbial abundances in an arable soil

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Since a few years biochar is discussed as a usable means to increase soil fertility, crop yield and for carbon sequestration in soils. The two main production processes for biochar production from plant residue feedstock are pyrolysis (pyrochar) and hydrothermal carbonization (hydrochar). Chemical and physical characteristics strongly differ between pyrochar and hydrochar potentially showing different effects on soil biota. In this microcosm study, we added pyrochar, hydrochar (unwashed and washed) and un-carbonized feedstock (Miscanthus) (2 % w/w) to a sandy arable soil and incubated these mixtures at 20°C and adjusted water tension (pF 1.8) for 240 days. Fluxes of greenhouse gases (GHG: CO_2, N_2O and CH_4) were measured and soil samples were analyzed for physicochemical (pH, Nmin, extractable organic C and N) and biological (microbial biomass, ^{13}Cmic, abundances of archaea, bacteria and fungi by qPCR) properties after 15, 30, 60, 120 and 240 days of incubation. We aimed to investigate the decomposition of the different amendments and that of the native soil organic matter to evaluate its C sequestration potential and to follow potentially connected temporal changes of the soil microbial community. We found that both hydrochar and the feedstock material increased CO_2 emissions, while showed no effect on N_2O and CH_4 emissions. Lowest CO_2 emissions and mineralization of the material itself were found in the pyrochar treatment, but N_2O emissions were highest in this treatment. Microbial biomass was strongly increased by hydrochar and feedstock material leading to N-immobilization. Fungal abundance (gene copy numbers of the fungal ITS region) increased during the incubation in both hydrochar treatments. Abundances of bacteria and archaea (gene copy numbers of specific 16S rRNA) were initially reduced in the hydrochar treatments, indicating the presence of inhibitory substances. In comparison to the untreated soil, pyrochar addition showed only weak effects on microbial abundances. We conclude that especially unwashed hydrochar influenced microbial abundances, without showing a strong reduction of GHG emissions in comparison to the feedstock material, pointing out the risk of hydrochar incorporation into soils. In contrast, reduced GHG emissions, lowest mineralization in combination with only minor effects on microbial abundances and community structure suggest that pyrochar could be beneficial to increase C-sequestration in arable soils.

Composition and activity of microbial community during decomposition of plant litter on two contrasting localities

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Decomposition of organic matter in soil is influenced by abiotic and biotic factors and their role is different depending on the site, organic substrate and its decomposition phase. Soil microbial community influences decomposition processed in different wayd, which are manifested, for example, by changes of microbial abundance and production of extracellular enzymes. The worked aimed in determining relationship between two main groups of soil microbial decomposers - fungi and actinobacteria.
The impact of selected factors on decomposition processes were determined by experiment with litterbags filled with litter of plant with different properties: Astragalus exscapus, Fagus sylvatica and Carex humilis. Litterbags with three plant species were placed under the litter layer at both sites. Litterbags and samples were collected every two months during years 2011-2012. Each litterbag was weighted, DNA was isolated from litter and soil samples and analyses of quantitative real time PCR with 16S/18S rRNA primers were performed. Enzymatic activities, element composition were also assessed in plant litter.
Results of the thesis showed substrate specificity of enzyme activities and differences between sites during decomposition. Overall, higher exocellulase enzyme activities were detected in Vienna, while higher endocellulase enzyme activities were detected in Oblík. Variable oxidase activities were detected at both sites. Soil microbial decomposer communities were typical by dominance of actinobacteria over fungi at both sites. Fungi proved to be a substrate specific microorganisms as opposed to actinobacteria that proved to be a local specific microorganisms. Both microbial groups correlated positively with each other. The experiment showed that actinobacteria use different type of enzymes or different strategy than fungi for heterotrophic growth.

The management system can influence the physiological function and social interaction of phosphate solubilizing bacteria isolated rhizosphere of Carica papaya L.!

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Agricultural practices can affect the soil environment, and consequently modify the dynamics of microbial communities. The soil microbial activity is essential for nutrient cycling processes and plant nutrition in natural conditions. Phosphorus (P) is an essential nutrient for plants, however, in most soils is available in form, it tends to be associated with iron oxides, aluminum and calcium. Thus, the use of microorganisms capable of solubilizing insoluble phosphate forms has been gaining momentum in the polls. The present study aimed to isolate bacteria in the rhizosphere of *Carica papaya* L., grown in organic and conventional planting system, assessing their ability to solubilize phosphate and verify that the type of management influence on functional cooperation in solubilize phosphate and bacterial growth. bacterial and physiological diversity. They were isolated from the rhizosphere of C. papaya, 5 phosphate solubilizing bacteria (BSF) of conventional cultivation system, and phosphate solubilizing bacteria 7 organic farming system. The largest free P concentrations, or extracellular, were found amid growing by bacteria isolated from the organic farming system. A cooperative behavior in cell growth was observed being independent source cropping system. However, compared to phosphate solubilization, it was not observed cases of physiological isolated cooperation between the conventional system. A positive physiological cooperation found in the interaction between bacteria of the organic system or conventional / organic mixture. The combined use of these bacteria as environmentally sustainable bioinoculantes is a viable alternative to promote the growth of C. papaya plants in tropical soils. The physiological cooperation in functionality to solubilize phosphate presented in some cases is extremely important to try to understand the behavior of these micro-organisms when used together as bioinoculantes.

Decomposition traits and enzyme production of saprotrophic fungi are shaped by the combination of their ecophysiology and taxonomy

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An extensive screening of saprotrofic fungi for their production of hydrolytic enzymes and laccase was performed. The study considered 111 strains of Basidiomycota, 39 strains of Ascomycota and 2 strains of Mucoromycotina belonging to wood decomposers that cause white-rot (WR) or brown-rot (BR), other wood associated saprotrophs (WA), litter decomposing cord-forming Basidiomycota (LDF), and saprotrophic microfungi (SA).The presence of enzyme-encoding genes was also analysed in the published genomes of saprotrophic fungi. Several genes, including those for acidic phosphatase, b-glucosidase and N-acetylglucosaminidase, were common in the genomes with enzyme activity widely displayed by fungi, while other enzymes, such as certain hemicellulases or laccase, were produced less frequently. Basidiomycota exhibited higher activities of all enzymes, except alkaline phosphatase, a-glucosidase, N-acetylglucosaminidase, a-mannosidase and a-fucosidase, than Ascomycota. The SA and BR fungi showed distinct enzyme production patterns, while the enzyme production by WR, LDF and WA was similar. Differences among species were typically reflected in the level of enzyme activity rather than in the absence of enzymes.

Soil bacterial diversity from different animal settlements in maritime Antarctica

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Ice-free areas in Antarctica contain different marine animal colonies. Sea animals, such as birds and mammals, play an important role in biogeochemical cycling by transferring nutrients from aquatic to terrestrial environment. Soils rich in animal depositions have generally high organic carbon, total nitrogen and total phosphorus, allowing high microbial activity. In this context, field measurements indicated that penguin and seal colonies are significant sources for atmospheric N_2O, CH_4 and CO_2; therefore, determining bacterial diversity in these soils would allow knowing bacterial assemblages involved in nutrient recycling and greenhouse gas emissions.

The goal of this work was determine the diversity of bacterial communities from different soil animal settlements in maritime Antarctica. Therefore, soil samples were taken from birds (penguins and kelp gulls) and mammals (elephant-seals and fur-seals) settlements at Cape Shirreff (Livingston Island). Subsequently, using TRFLP profiles and pyrosequencing, structure and composition of edaphic bacterial communities were assessed, respectively. In addition, genes related to nitrous oxide emission and consumption were quantified. Finally, edaphic variables such as pH, organic matter, moisture, phosphorus, nitrate and ammonium contents were measured.

A lesser bacterial diversity in soils from sea-animal settlements, compared with control soils, was observed; in particular from penguins, in which also high contents of nitrogen, phosphorus and organic matter were determined. Conversely, soils from fur-seal colonies were more similar to the controls, may be due to the lesser sedentary behavior of these sea-mammals compared to the rest. On the other hand, bacteria belonging to Gammaproteobacteria dominated soils influenced by animals, in particular those belonging to Xanthomonadaceae family; besides, a high abundance of members of phyla Actinobacteria and Bacteroidetes were found. In agreement with this, high abundances of *nirK* and *nosZ* genes belonging to members of Xanthomonadaceae family were observed.

These results suggest that soils influenced by animals promote specific groups of bacteria, which would have an important role in the recycling of nutrients in Antarctic terrestrial ecosystems. Financial source: INACH project RG_14-14.

Carbon and nitrogen co-metabolism and microbial nitrogen-mining both determine the extent of plant material decomposition in four Australian pasture soils.

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A better understanding of the interaction between nitrogen (N) availability and plant material decomposition is necessary to predict how agricultural management practices or atmospheric N deposition may affect soil organic carbon (SOC) stocks. Several hypotheses as to how N addition affects decomposition of soil organic matter exist: a) inhibition of micro-organisms involved in lignin degradation; b) alteration of the abundance of copiotrophic vs oligotrophic micro-organisms; c) N limitation driving C decomposition due to microbial N-mining; and d) C and N co-metabolism is necessary for decomposition due to cellular stoichiometry. We evaluated the extent of plant material decomposition in response to N addition in four pasture soils varying in physico-chemical properties. We monitored microbial respiration of ^{13}C pulse-labelled buffel grass (*Cenchrus ciliaris* L.), wheat (*Triticum aestivum* L.) and lucerne (*Medicago sativa* L.) over a 365-day period. A weighted three-compartment mixing model was used to estimate soluble and insoluble respired plant-derived 13C (mg C kg^{-1} soil). Decomposition was primarily explained by plant species ($r2 = 0.72$; $P = < 0.001$). Total plant material decomposition followed the alkyl C: O-alkyl C ratio of the plant material (0.65, 0.12 and 0.24 for lucerne, buffel grass and wheat, respectively) as determined by solid-state ^{13}C nuclear magnetic resonance spectroscopy. Principle components regression analysis indicated that 26% of the variability of decomposition was explained by soil physical and chemical properties ($P = 0.001$). Decreasing C:N ratio consistently led to increases in the extent of plant material decomposition across the four soils, in agreement with carbon and nitrogen co-metabolism. However, N availability also played a plant-specific role with low soil N (< 0.07%) leading to increased decomposition of soluble buffel grass C and increasing N leading to decreased decomposition of insoluble lucerne C. This was consistent with microbial N-mining. These results indicate that multiple mechanisms affect microbial soil C cycling at any one time, and to varying degrees.

Evolutionary aspects of atromentin synthesis genes in Agaricomycetes

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Atromentin synthesis plays a central role in the secondary metabolism of many mushroom forming fungi, since atromentin is the precursor for many other secondary metabolites such as involutin. Many of the metabolites produced by atromentin have diverse functions such as participation in litter or wood decomposition through non-enzymatic mechanisms (e.g. Fenton reaction). It has been shown that at least two genes participate in atromentin synthesis including an aminotransferase (*atrD*) and an atromentin synthase (*atrA*), which is a non-ribosomal peptide synthase. We examined the presence of homologs from the two genes across published Agaricomycete genomes, their placement on those genomes and the phylogenetic relationships of the homologs for each gene respectively. Our results suggest that *atrD* homologs belong in a large aminotransferase gene family, but closely related *atrD* homologs are widespread mostly in Boletales and Polyporales. On the other hand, *atrA* is restricted in the Boletales, where is found in multiple copies and the distantly related agaric *Omphalotus olearius*. The distribution of *atrA* and *atrD* homologs on the genomes, shows that for all species most frequently one *atrA* homolog is in close proximity with one of the *atrD* homologs present on the genomes, suggesting that synteny for the two genes is at least partly preserved. The placement of *atrA* and *atrD* homologs from *O. olearius* on the corresponding phylogenies suggests that the presence of *atrA* and *atrD* in close proximity on the genome predates the diversification of Boletales. We have also detected a third gene that is always found in the vicinity of *atrA* and *atrD*, only when the two genes are found in the same genomic area. The gene is restricted in Boletales, *O. olearius* and *Piloderma croceum*, its closest homologs are found in Bacteria and encodes a potential quinone oxidoreductase, suggesting a possible recruitment of the enzyme in a function related to atromentin synthesis or subsequent metabolic steps.

Spatial heterogeneity of decomposition and fungal community composition within single *Quercus petraea* leaves

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In forest ecosystems, decomposition of litter is limiting step of carbon cycling and an essential process leading towards soil formation. This process is carried out mainly by fungi, which possess effective degradative enzymes. Forest soils show considerable spatial heterogeneity of enzyme distribution as well as fungal community composition. Diversity of litter decomposers was described at different levels, from metres to kilometres, and in this work we asked if this spatial heterogeneity can be seen even at a scale of an individual leaf. To link fungal community composition to decomposer activity, we analysed fungal DNA in decomposition hotspots with high exocellulase activity and in nonactive patches. Litter was collected at different stages of decay in a *Quercus petraea* forest - leaves before litterfall and after 2, 10 and 22 months of decomposition. We measured the activity of exocellulase over the leaf surface and areas with the high and low enzyme activity were identified in each leaf. These areas of approx. 1 cm^2 were cut out, DNA was extracted and fungal community was analysed using ITS2 sequencing. Exocellulase activity varied considerably over the surface of a leaf (up to 20-fold) and the highest activities were detected after 10 and 22 months of decomposition. In one spot we found in average 42 genera, ranging from 20 to 68, what gives evidence for substantial diversity even at such small area as 1cm^2. Community of active and nonactive areas within a single leaf were different, but not in a consistent way. There was, however, a clear shift in community composition across litter of different stages of decay. Early stages were dominated by endophytes, saprotrophic basidiomycetes increased with time. Our results show that fungal communities are spatially heterogeneous even at the level of individual leaves.

A comparison of methods for measuring the efficiency of microbial metabolism

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Microbial efficiency, or the proportion of metabolized carbon that results in biomass synthesis, is a critical step in terrestrial carbon cycling that determines the dynamics of soil organic matter pools and ecosystem carbon dioxide flux. Although recent reviews have drawn attention to the wide range of efficiency estimates commonly reported and the most likely underlying biological mechanisms, numerous methodological concerns persist. Efficiency estimates diverge when compared among communities and habitats (e.g., ~0.3 for aquatic and ~0.6 for soil environments) but the techniques used in these systems are also very different. As a result, the variability in efficiency estimates produced by such distinct methods is not necessarily of ecological relevance until inherent differences among methods have been fully explored. Here we describe a methods comparison that utilizes both traditional measures of microbial efficiency (e.g., substrate-based, biomass-based) and emerging techniques (e.g., growth rate-based, calorimetry) to track how efficiency varies over short (hourly) and long (daily) periods of observation. Control (untreated) soil from the Harvard Forest Long Term Ecological Research site in Massachusetts, USA, was amended with ^{13}C-glucose and ^{18}O-water in laboratory mesocosms and monitored for changing rates of soil dissolved organic carbon (DOC) uptake, respiration (R), microbial biomass (MB) production, DNA synthesis, and heat (Q) flux. These measurements allowed calculation of four different microbial efficiency estimates: 1) (?DOC – R)/?DOC (substrate-based), 2) ?^{13}C-MB/(?^{13}C-MB + R) (biomass-based), 3) ?^{18}O-DNA/(?^{18}O-DNA + R) (growth rate-based), 4) Q/CO_2 (energy-based). Preliminary trials based on a small sample size have indicated that energy-based efficiency decreases with depth at Harvard Forest ($p = 0.10$), similar to the results from substrate-based techniques in other systems. Our methods comparison will clarify the effects of numerous time-dependent controls over microbial efficiency such as biomass turnover and substrate recycling. These efforts will increase the transparency of microbial efficiency methods for researchers looking to choose the most appropriate technique for their scale of inquiry.

Uniformly ^{13}C-Labelled Biomass Tracers: Advances in ^{13}C-Techniques tracing changes in soil microbial processes and populations

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Uniformly ^{13}C-Labelled Biomass Tracers: Advances in ^{13}C-Techniques tracing changes in soil microbial processes and populations.

Stable isotopes like ^{13}C and ^{15}N have been used in ecology to trace microbial activities in organic environments like natural and agricultural soils. They are used to quantify C & N fluxes and transformations in complex ecosystems where decomposition processes of soil organic matter (SOM) are driven by soil microbial populations.

Since 2005, IsoLife produces a large range of ^{13}C labelled plant materials - from 1.2% up to 98 atom % ^{13}C. They have opened up new ways in unravelling key processes and functional relationships and studying decomposition of plant residues and formation of soil organic matter. Their application also improved the sensitivity of analytical techniques such as Stable Isotope Probing (SIP).

Recent advances in soil ecology

i) SIP enables the detection of functional organisms in complex ecosystems at species level using U-^{13}C substrates and density gradient centrifugation to separate ^{12}C- from ^{13}C-DNA or -RNA. Subsequent sequencing of the ^{13}C-bands reveals the functional species active in decomposition of polymers like chitin, cellulose, lignin, etc. This technique yields valuable information especially in complex soil ecosystems with high background ^{12}C. Trophic interactions in food webs, biodiversity issues, carbon fluxes in symbiotic associations, and formation of SOM can be studied with a renewed focus.

ii) Methods for studying in situ ecological relations enable tracing C and nutrient transfer between hosts and symbiotic organisms or the utilisation of cellulose-^{13}C by single cells in soil. These can be measured and visualised by microscopy techniques such as high resolution imaging mass spectrometry (NanoSIMS) by using ^{13}C-labelling at extreme enrichment levels (>97 atom %).

iii) Decomposition studies have been carried out with partially or uniformly ^{13}C-labelled plant parts (whole leaves or roots; intact or ground) or extracted plant materials like (hemi)cellulose yielding detailed information about fractions respired, organic matter transformations, and distribution among different SOM fractions.

iv) Atmospheric labelling in m^3 volumes with $^{15}N_2$ to demonstrate the presence of N_2 fixation.

The poster shows an overview of the achievements during the last 3 years of using in situ uniformly ^{13}C-labelling and uniformly ^{13}C-labelled substrates in soil ecology.

Effects of plants on the structure, function and diversity of bacterial communities

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Microbial communities in soil and litter are known to be affected by aboveground vegetation. While the relations between fungi and plants are frequently described, the relations between bacteria and plants are less explored. Bacteria are important in soil and litter because they are involved in the decomposition of plant biomass, they can act as pathogens, roots symbionts or opportunists using root exudates. Individual plant taxa create specific niches, such as rhizospheres or litter with varying chemical composition. There are some studies describing the link between specific bacteria and living plants or their dead biomass but the effect of diversity of plants on bacterial communities remains unclear. The aim of this work is to describe the diversity of bacterial community in the context of plants diversity in forest sitesin the Bohemian Forest National Park, Czech Republic. The 60 sampling sites represent a gradient of tree diversity (1-5 species) and the diversity of ground vegetation (1-45 species). We hypothesize that the diversity and evenness of bacterial community composition increases with increasing plant diversity. Bacterial comnunities in soil and litter will be compared by sampling 8 soil cores per sampling site, each taken in the distance 2m from a dominant tree. The composition of bacterial community will be characterized by 16S rRNA sequencing and the composition of vegetation in samples will be characterized by TrnL(F) sequencing of plant roots on the Illumina MiSeq platform. Further, the diversity of bacterial community will be also compared in the roots of *Vaccinium myrtillus*, *Calamagrostis* and *Picea abies* growing at sites with different plant diversity. The project should contribute to the understanding of the relationship between diversity and activity of plants and between diversity and functioning of communities of bacteria.

Evidences on the ability of mycorrhizal genus *Piloderma* to use organic nitrogen and deliver it to Scots pine

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Boreal forest soils are often regarded as nitrogen (N) poor as the forest growth is often limited by N availability. However, it has been shown that it is not the N pool size but the form of N compounds that restrict N availability to plants and other organisms. Large part of N in boreal forest soil is bound to soil organic matter (SOM) and N is in recalcitrant organic forms that need to be degraded before N is available for plants and other organisms. Ectomycorrhizal fungal (ECM) symbiosis with forest trees has been proposed to link plant photosynthesis and soil organic matter (SOM) decomposition through the production of fungal enzymes which promote SOM degradation and nitrogen (N) uptake. However, laboratory and field evidence for the existence of these processes are rare.
We studied *Piloderma* sp., a common ECM genus in boreal forest soil, as a model mycorrhiza to investigate the interactions between plant, ECM fungi and organic N uptake in a series of studies ranging from laboratory to field scale. The abundance of *Piloderma* sp. was studied in root tips and soil over one growing season and in winter. Protease production was measured from ectomycorrhizal root tips and soil solution in the field and also from pure fungal cultures. The field data on *Piloderma* was compared to forest productivity and environmental factors, obtained in the SMEAR II forest research station (https://www.atm.helsinki.fi/SMEAR/index.php/smear-ii) where the field study was conducted. We also tested the effect of *Piloderma olivaceum* on host plant organic N nutrition in the laboratory.
The results showed that *Piloderma* sp. was highly abundant in the field and produced extracellular proteases in the field, which correlated positively with the gross primary production, temperature and soil respiration. In the laboratory, *Piloderma olivaceum* could improve the ability of *Pinus sylvestris* L. to utilize N from extragenous proteins. As earlier studies have shown that *Piloderma* sp. produces SOM oxidizing laccase enzymes, we suggest that ECM fungi are important in forest C and N cycling due to their ability to access proteinaeous N in SOM. As *Piloderma* sp. abundance seemed to be seasonally highly variable, recycling of fungal-bound N after hyphal death may therefore be of primary importance for the N cycling in boreal ecosystems.

Ecology and diversity of oxalotrophic bacteria: an in silico analysis

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Oxalate, the simplest dicarboxilic acid, is present in various and diverse environments such as soils and mammalian gastrointestinal tracts. Oxalate can also form insoluble precipitates with metal ions (e.g. calcium, magnesium). Oxalotrophy, the ability to use oxalate as a carbon source, is mainly the result of bacterial catabolism, which can be either aerobic or anaerobic. Two main enzymes, namely formyl-Coenzyme A (CoA) transferase (EC 2.8.3.16) and oxalyl-CoA decarboxylase (EC 4.1.1.8) encoded by the *frc* and *oxc* genes, respectively, are known to be involved in oxalotrophy. An in silico approach was used to explore the taxonomic distribution and the phylogenetic diversity of the oxalotrophic bacteria across biomes. Sequences and metadata of 3481 FRC and 4540 OXC proteins were extracted from the NCBI database. Additionally Gribskov protein profiles were created to explore 2774 completed bacterial genomes and various metagenomes. Subsequently, diversity, taxonomic, phylogenetic and genomic analyses were performed. In terms of diversity, amino acid based OTU clustering revealed that OXC total diversity was more constrained than FRC. Regarding the taxonomic and ecological distributions, bacterial genomes containing both *frc* and *oxc* genes were restricted to three phyla namely Actinobacteria, Firmicutes and Proteobacteria and originated from terrestrial, aquatic and clinical environments. At the genomic level, the number of copies of the *frc* gene was highly variable compared to *oxc*. The existence of a *frc-oxc* operon, which was located on chromosomes or plasmids, was detected in 33 genomes. However, numerous genomes contained only one of the two genes, raising the question of their functional role *in situ*. Finally, metagenome analyses indicate the presence of this pair of genes in forest soils and in the gut of termites, highlighting the role of oxalotrophic bacteria in carbon cycling notably in the case of the oxalate-carbonate pathway.

Fungal functional diversity and enzyme activity patterns in decaying logs of 13 temperate tree species in an in situ decomposition experiment

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Wood-inhabiting fungi play crucial roles in deadwood decomposition. While the chemical processes of wood decay are well understood, little is known about which factors explain the heterogeneous distribution patterns of fungi and their corresponding enzyme activities. We therefore investigate spatial and temporal variations of fungal communities, functional gene diversity and enzyme activities in an in situ long-term deadwood experiment (BELongDead), which was designed and launched within the German “Biodiversity-Exploratories” in 2008 to follow the decomposition processes during the coming decade. It includes logs of nine deciduous and four coniferous tree species replicated across 30 differently managed forests plots in three distinct areas across Germany.
A recent study conducted on the same experimental platform revealed that decomposition rates and the activities of lignin-modifying enzymes (in deadwood under natural conditions) were controlled by the succession of in the fungal communities and competition scenarios rather than by fungal OTU richness.
To link fungal community structure with microbial-mediated ecosystem functions and processes we use state of the art molecular techniques (Next Generation Sequencing) in combination with a sophisticated set of enzyme assays to measure lignocellulolytic hydrolases (e.g. b-glucanase, cellobiohydrolase, endocellulase, xylanase, b-xylosidase) and oxidoreductases (e.g. laccase, Mn-dependent and Mn-independent peroxidases) to I) identify and quantify wood decomposition changes under different forest managements, II) to analyze fungal community assembly processes and the mediated metabolic fungal activity on transcript- and enzyme- levels, and relate them to changes in wood chemistry and decomposition of the different tree species.
First results of a 2012 sampling revealed a significant impact of deadwood species and forest management intensity on the fungal community structure. Enzymatic activities displayed big differences between deciduous and coniferous tree species, which relies to different fungal ecologies.

Enzyme activities of aerobic (hemi)cellulolytic bacteria isolated from Algerian soils and compost

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Identification of bacteria for the production of carbohydrolytic enzymes is extremely important given the increased demand for these enzymes in many industries. In recent years, increasing attention has been devoted to lignocellulose decomposing enzymes involved in many biotechnological processes that includes bioethanol production, pulp, paper industry and fiber refining. Algerian compost and different soils were targeted to study the potential of different enzymes involved during the biodegradation process.
All isolates were initially screened for cellulose production based on a qualitative screening using agar plate assays with carboxymethyl cellulose as carbon source. The selected strains were then subjected to a second quantitative screening step measuring the endocleaving enzymes endo-1,4-β-glucanase and endo-1,4-β-xylanase. Isolates were further tested for their ability to produce the following enzyme activities from wheat straw : cellobiohydrolase, β-glucosidase, β–xylosidase, β–mannosidase, β-galactosidase, arabinosidase and other polysaccharides degrading enzymes: α-glucuronidase, α-glucosidase, acid phosphatase and chitinase. Upon initial screening, it appeared that most of the isolates were able to develop clear zone surrounding colonies indicating cellulase production. However, in the liquid medium, isolated strains showed high variation in their ability to produce cellulase and hemicellulase. Different enzymes were produced on wheat straw, indicating that they can have capacity of producing multiple extracellular enzymes. However, some isolates are able to attack only amorphous cellulose and their decomposition on lignocellulose is rather negligible. Based on 16S rRNA gene, the isolated genera belonged to phyla Proteobacteria and Actinobacteria.
The results confirm the potential importance of bacteria in (hemi)cellulose degradation. However, the enzymatic activities may considerably differ from species to species. The active bacterial strains may have potential in industrial applications, such as pretreatment of lignocellulosic material or enzymatic hydrolysis improvement through the preparation of optimized enzyme–cocktails.

Cellulose was decomposed faster in fallow soil than in meadow soil because of a quicker start of the process

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Fallows and meadows are two semi-natural agrobiocenozes with similar plant biomass but differing in plant community composition and diversity and in the ecosystem time-span and succession level. To discuss whether the two types of ecosystem would differ in their functioning we compared cellulose decomposition in soils from six fallows and six meadows in one area. From our previous studies on the same plots their soil physico-chemistry, plant community composition and microbial community structure were known. The cellulose decomposition was measured with the method of Grunda (1967). During 10 weeks of observations in the laboratory conditions decrease of cellulose strips was estimated. We developed a model allowing for a lag time in decomposition and a non-zero asymptote. The model showed that a longer lag phase was responsible for lower efficiency of cellulose decomposition in meadows in comparison to fallows, even if decomposition rates and asymptotes were potentially similar in both ecosystem types. The fallow soils had higher contents of available NPK, which might have favoured microbial activity. In addition, the studied fallows were farmlands abandoned without management with dead organic matter remaining on the field every season, which might have selected cellulolytic organisms in this type of ecosystem.

Fungus-Bacteria Interactions in Decomposing Wood

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Fungi are the principal organisms responsible for the breakdown of wood, where they display highly competitive and territorial ecological strategies. Bacteria are also ubiquitous in wood, yet their relationship with wood-decay fungi is still largely unknown; there is potential for competitive, mutualistic or facilitatory relationships. Previous studies suggest that the bacterial community in wood varies according to the dominant fungal species present, but these studies have been restricted in scope and/or methodology. We present the results of a large-scale field experiment and survey of bacterial diversity in wood that investigates how the bacterial community is influenced by the fungi present. Wood disks were lab-colonised by one of three wood-decay basidiomycetes (*Vuilleminia comedens*, *Trametes versicolor* and *Hypholoma fasciculare*) and left in the field for one year at six sites across the southern UK, along with sterile control disks. ITS2/16S rRNA gene amplicon sequencing was then used to identify the fungal and bacterial communities in the samples. Using this approach allows us to link bacterial identity with that of the fungi present at the time of sampling and in the past, as well as to explore spatial effects.

Detection of organotin compounds and degradation by litter-decomposing fungi

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Organotin compounds, especially tri-substituted tin derivatives such as tributyltin, exhibit highly toxic characteristics and endocrine disrupting activities. They have been used in antifouling paints on ship hulls for several decades and are found in sediments. In addition they are still in use for biocidal purposes in agriculture. Reduction of toxicity is associated with dealkylation and dearylation. However, without any treatment these processes have with half-lives of several years. Litter-decomposing fungi produce powerful enzymes that degrade recalcitrant plant lignin and also a variety of persistent chemicals in nature, and these fungi can be easily added to contaminated soil.
In order to detect the endocrine disrupting activities of organotin compounds a bioluminescent yeast strain has been equipped with a plasmid carrying a hybrid retinoic X receptor. This bioreporter was found sensitive enough to detect the organotin compounds tributyltin and triphenyltin at nanomolar concentrations and was also successfully tested in untreated spiked sediment samples (Kabiersch et al. 2013).
Furthermore, a set of fungi was screened for the ability to grow on agar plates containing tributyltin and to grow into sediment spiked with tributyltin. Two litter-decomposing fungi were chosen for further degradation studies, in which the removal of endocrine disrupting activities (determination by the bioluminescent yeast assay) and the formation of metabolites (determination by GC-MS) will be followed.
Reference: Kabiersch et al. (2013) Analytical Chemistry 85: 5740-5745

Efficacy of biochar and compost on remediation of copper contamination in vineyard soils - effects on soil microbiology

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Copper (Cu) enrichment in vineyard topsoils has been caused by the application of Cu-based fungicides over a period of more than a century. This has resulted in soil Cu-concentrations significantly exceeding biological effect levels. A longer-term mitigation strategy to reduce the copper bioavailability in topsoil layers of vineyard soils is of key importance as there are currently little alternatives for copper in plant protection in organic viticulture. A promising approach to reduce bioavailability of Cu and thus improve the soil as a habitat for microorganisms is the addition of organic soil amendments like biochar (BC) and compost.
In this study a greenhouse pot experiment was conducted. Two soils from different vineyards in Austria (varying in texture, pH, soil organic matter and Cu-content) were treated with 12 combinations of biochar compost amendments at application rates equivalent to 10 - 80 t ha-1. Grape vine seedlings and cover crops (a mixture of legumes and non-legumes) were planted in the pots. The pots were incubated for more than a year with 3 samplings throughout the incubation period. Microbiological parameters such as potential extracellular enzyme activities (exoglucanase, ß-glucosidase, exochitinase, phosphatase, protease, urease, phenoloxidase and peroxidase activity), basal respiration, PLFAs, and pH-value, NO_3-N and NH_4-N were measured.
Microbial reactions to additives were soil-specific. In summary, our results indicate that no treatment consistently influenced soil microbial activity but showed a tendency of highest microbial activities for soils amended with acid-activated BCs and significantly changed microbial community.

Fungal research on an artificial deadwood decomposition experiment in the German Biodiversity Exploratories

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A substantial part of terrestrial carbon is bound in wood. After die-back, this source is continuously degraded by microorganisms, hereby structuring forest ecosystems and influencing multiple ecosystem functions, like carbon sequestration, nutrient cycling, and habitation of wood-dwelling organisms. However, detailed joint in situ analyses of microbial communities, enzymatic processes and complex changes in physicochemical wood parameters during the course of wood decay are still largely lacking. In our research consortium we follow questions like:

- How does decomposition of 13 common Middle European tree species proceed?
- How does forest management influence microbial communities and respective decomposition processes?
- Who are the key species and what are their characteristics?

The German Biodiversity Exploratories (DFG Priority Programme 1374) were established 2006 in three larger regions (Biosphere Reserve Schorfheide-Chorin, National Park Hainich, Biosphere Reserve Schwäbische Alb) aiming to understand the role of land use and management for biodiversity and in turn ecosystem processes. In late 2008 until beginning of 2009, an experimental platform for researching wood decomposition was established, called the BELongDead experiment. Freshly cut logs (diameter of 30–40 cm and 4 m length) of 13 temperate tree species (*Acer, Betula, Carpinus, Fagus, Fraxinus, Larix, Picea, Pinus, Populus, Prunus, Pseudotsuga, Quercus* and *Tilia*) were placed in three fold replication in 30 representative research plots (unmanaged and managed beech forest and managed conifer forest) to follow the decomposition processes during the coming decade.
In our research consortium we follow the succession of fungi (fruiting body observation and molecular analyses of the wood), their enzymatic activity (cellulolytic, hemicellulolytic and nitrogen cycling-related hydrolases, and ligninolytic oxidoreductases) and multiple wood physico-chemical parameters (e.g. lignin content, extractives, element content, pH, etc.). To understand the underlying molecular mechanisms, we aim at analyzing the expressed fungal enzyme-encoding genes as well.
Our gathered information will be merged and correlated with data from other research groups working on the same decomposing logs, for example data on respiration, occurrence of arthropods and carbon and nitrogen fluxes. Altogether, we expect to come to a more detailed understanding of wood decay in temperate forest ecosystems.

The influence of traditional agriculture on soil organic matter in tropical ecosystems of Papua New Guinea

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The research concerns the influence of traditional agriculture on soil organic matter (SOM) balance in lowland rainforests of Papua New Guinea. The plots in the vicinity of Wanang represent a suitable location. The plots were formerly deforested, burned off and cultivated under traditional agriculture. Some of these plots were abandoned and the rest of plots is still cultivated. The succession was initiated in different time period. Within the previous research the vegetation development was described on these plots, particularly the change in attributes of leaves of dominant tree species. The plots represent unique chronosequence allowing real-time observation of tropical ecosystem development. In our study we are focused on SOM and related soil properties, litter properties and its decomposition and condition of microbial communities. By this approach we describe dynamic of SOM and we determine main influencing factors. We hypothesize there applies the effect of soil degradation typical for tropical agriculture. It is usually connected with the decrease of SOM and its ecosystem services. Considering the small scale of disturbances, the effects will be of lesser importance compared to modern agriculture.

The effect of soil fertility on fungal communities, enzyme activities and soil carbon dynamics in unmanaged forests

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The boreal forest is one of the largest biomes in the world and constitutes a persistent terrestrial carbon sink with a major fraction of the carbon stored in the soil. Mycorrhizal and saprotrophic fungi are dominant components of the boreal ecosystem and play important, yet fundamentally different, roles in nutrient and carbon cycling. Saprotrophic fungi are considered to be the main wood and litter decomposers, while mycorrhizal fungi mine for nutrients in more processed soil organic matter. By producing extracellular enzymes mycorrhizal fungi may act to decrease organic matter stocks, but may also contribute substantial amounts of organic matter in the form of mycelium.
In this study we aim to investigate the interplay between soil fertility, fungal communities, enzyme activities and soil carbon dynamics in unmanaged boreal forests. In order to investigate influences of fungal communities on organic matter dynamics across a soil fertility gradient, stable isotopes, C:N ratio, ergosterol and extracellular enzyme activities were measured throughout fine-scaled soil depth profiles. We found that organic matter was more actively processed on nutrient rich sites, while divergent C:N, ^{14}C and ^{15}N depth gradients indicated impaired decomposition and nitrogen retention on nutrient poor sites. Higher activities of manganese dependent peroxidases on richer sites correlated with lower stocks of total organic matter, supporting that more rapid decomposition lead to lower carbon sequestration.
Clear shifts in fungal community composition was observed both along soil depth and fertility gradients. Soil fertility, enzymes activities, carbon stock and dominant tree species were significant environmental predictors. The results suggest that fungal communities play a regulatory role in carbon dynamics through a nutrient gradient in unmanaged old growth forest by facilitating humus accumulation on nutrient poor soils and actively recycling organic matter on richer sites.

Fungal extracellular enzyme activity and biomass in coarse woody debris of 13 tree species in the early phase of decomposition.

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The decomposition of coarse woody debris (CWD) is predominantly mediated by fungi through their extracellular enzymatic activity. While the decomposition rates and changes in physico-chemical parameters of CWD have been investigated in many studies, data on in situ enzymatic activities which are related to fungal biomass are rare. We also lack information of differences in fungal activity and decomposition processes in sapwood and heartwood of common tree species in German temperate forests.
This study is part of an artificial long-term dead wood experiment (BELongDead) which was designed and launched within the experimental platform of the German “Biodiversity-Exploratories”. Samples of sapwood and heartwood were taken from logs of 13 tree species (*Acer, Betula, Carpinus, Fagus, Fraxinus, Larix, Picea, Pinus, Populus, Prunus, Pseudotsuga, Quercus* and *Tilia*; n=3) that were exposed for 6 years on the soil in a European beech forest at the Nationalpark Hainich, Germany. We established spectrophotometric and HPLC-methods to detect a multitude of fungal extracellular enzymes involved in carbon and nitrogen cycling, for example: i) endocellulase, cellobiohydrolase, b-glucanase, xylanase, b-xylosidase involved in (hemi)cellulose decomposition; ii) laccase, Mn-dependent and Mn-independent peroxidases involved in lignin decomposition, and iii) chitinase as well as peptidase as proxy for N-cycling processes. CWD samples were analyzed for ergosterol as a proxy for fungal biomass and the contents of C, N, lignin and soluble compounds were determined.
The fungal biomass was significantly larger in sapwood than in heartwood and larger in deciduous than in coniferous species. Fungal biomass correlated positively with the N content of CWD and, in heartwood, negatively with acetone extractives. Enzyme activities were higher in deciduous than in coniferous CWD and higher in sapwood than in heartwood, which corresponded to the general life strategies and ecologies (e.g. white rot vs. brown rot) of fungi. However, correlations between enzyme activities and ergosterol content were generally weak, which calls for more detailed analyses on the drivers of enzymatic activities in CWD, including fungal communities. Nevertheless, our data on fungal biomass and enzymatic activities confirm that in the initial state, the decomposition of deciduous CWD is faster than of coniferous CWD under comparable conditions.

Studies on biodegradation of naphthalene and anthracene by *Aspergillus glaucus* strain isolated from Antarctic soil

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There is an apparent consensus within the scientific literature that refers to processes associated with bioremediation on the need to develop eligible approaches for purification of polluted water and soil on the basis of the microbial capacity to degrade a large variety of xenobiotics. Polycyclic aromatic hydrocarbons (PAHs) are one of the wide-spread pollutants in the environment. A variety of microorganisms have been described to play a role in PAHs degradation. Exposure to PAHs increases health risk for people and other living organisms.
The degradation of naphthalene and anthracene by the strain *Aspergillus glaucus* AL1, isolated from Antarctic soil was studied in the present work. The gradual reductions of concentrations of investigated PAH compounds included in the medium as sole carbon sources were followed by GC-MS analyses. The enzyme analyses for phenol hydroxylase and catechol 1,2-dioxigenase activities were performed. The values obtained for the activity of both enzymes when cultivating the strain in medium including each of the two compounds individually are quite similar. For example, in the process of naphthalene degradation, phenol hydroxylase is 1,48 U/mg protein and catechol 1,2-dioxigenase activity is 0,24 U/mg protein, while in the experiments conducted with anthracene, the activities obtained are respectively 1,12 U/mg protein and 0,16 U/mg protein on the fifth day of culture. The catabolic gene encoding the enzyme with catechol 1,2-dioxigenase activity which is crucial for the aromatic catabolism was sequenced.

Microbial communities' fungal to bacterial dominance alters carbon cycling in soil

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Soil microbial communities' fungal:bacterial (F:B) ratios are often thought to be associated with the carbon (C) storage potential of soil systems, though the mechanisms underpinning observed relationships are yet to be fully explored. We sought to specifically evaluate whether soils with different F:B ratios show altered functional profiles which may lead to differences in C storage using metatranscriptomic, metaproteomic and isotope tracer techniques. A mesocosm experiment was performed using two soils which were almost identical in their physico-chemical properties but differed in their microbial community structure, and specifically their F:B ratio (assessed by PLFAs). To assess short term C storage, ^{13}C labelled foliar litter was added to root-free soils and traced into respired CO_2 and microbial biomass. Litter derived ^{13}C in respired CO_2 was consistently lower and the residual ^{13}C in bulk soil organic matter a month after litter addition was significantly higher in high F:B soil both indicating higher C storage potential in soils with fungal dominance. Phylogenetic information from RNA sequencing and protein profiling confirmed fungal dominance in high F:B soils; and also identified fungal phyla increased in abundance on litter addition. On the contrary, most bacterial phyla did not shift on litter application with the exception of Actinobacteria. Actinobacteria: rest of bacteria (A:RB) ratio increased significantly in low F:B soil suggesting they take up the role of fungi in litter degradation. To gain a mechanistic processes understanding, we focussed on the genes involved in the central C metabolism pathways (glycolysis, pentose phosphate pathway, TCA and associated cycles). Only the TCA cycle gene transcripts demonstrated a discernable trend; citrate synthase, aconitase, isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase significantly increased in abundance in high F:B soils. Although the TCA cycle yields no net C assimilation, increased abundance of several genes shuttling its intermediates into biosynthetic pathways suggests a higher cataplerotic flux of TCA cycle in fungal dominated communities indicating an efficient substrate metabolism. In conclusion, a higher litter C assimilation to respiration ratio of microbial communities, higher amount of litter derived soil organic C and transcript indicators of biosynthesis-driven C metabolism clearly implicate fungal dominated communities in soil organic carbon formation.

Towards linking fungal genes to chemical spectra from soil organic matter using machine learning

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Characterization of biological and chemical processes in soil organic matter (SOM) are increasingly being done by complementary high-throughput experimental techniques. Interpreting these diverse high-dimensional data types together poses challenges about data integration and analysis. A bioinformatics merge of machine learning and chemometrics may be a promising avenue to fully explore links between biological and chemical processes.
Here, we propose computational methods for linking genes to decomposition mechanisms by integrating genome-wide transcription profiling data (RNA-Seq) with chemical changes in the organic compounds occuring during SOM decomposition as measured by chemical spectra (FT-IR, pyrolysis-GC/MS). The methods are intended to enable extraction of patterns that can be recognized and interpreted by domain experts.
We also wanted to show proof of concept of integration of genome-wide gene expression data with chemical spectra from a study of two fungal species during SOM decomposition. This experiment was set up to measure effects of decreasing glucose levels over time on the decomposition of SOM. Two ectomycorrhizal fungi *Paxillus involutus* and *Laccaria bicolor* were grown in axenic cultures on SOM water extracts in a time series experiment.
Species-specific co-expression networks were constructed from pairwise correlations between gene transcriptions across the four time points. Genes of similar functions between fungi were identified from orthology detection and spectral associations akin to chemometric 2D synchronous correlation analysis were computed and correlated with gene transcriptions to create gene-spectral association networks.
Cross-species clustering was performed for discovery of conserved as well as species-specific modules of co-expressed genes and including correlated spectral patterns thus enabling characterization of the decreasing glucose responses in terms of modules of genes and associated spectral ranges.
Modules of genes with similar expression profiles across time points were found and linked to certain spectral ranges of the FT-IR chemical spectra as well as certain compounds found with pyrolysis.
These results demonstrate a powerful application of machine learning clustering tools for integration of transcriptomics and chemical spectroscopy to leverage interpretation of data from controlled fungal SOM experiments.

Structure of microbial communities in the environmentally exposed construction wood samples of different species

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Although the biodegradation of wood is a positive feature considering the environment, it is a problem when wood is used for construction and commercial applications. Since the primary wood decomposers are fungi, limiting their growth is one of the main goals in preventing biological wood degradation. The second important decomposers that might influence the industrial wood lifespan are bacteria.There are several methods for wood protection against the fungal and possibly bacterial decay that are based either on limiting the humidity or the use of biocides for wood impregnation. Use of the resistant tree species might also be beneficial, but these are in deficit in most of the Europe.
Our main goals were (i) to compare the structure of fungal and bacterial communities on the surface and inside the samples taken from different tree species (i.e. Douglas fir (*Pseudotsuga menziesii*), western red cedar (*Thuja plicata*) and Norway spruce (*Picea abies*); and (ii) to profile the fungal and bacterial communities on the surface and inside the spruce samples that were thermally modified or chemically treated (ACQ - alkaline copper quaternary solution - Silvanolin©).
The surface and inside of wood samples were mechanically processed in order to obtain homogenized wood meal that was used as starting material for DNA extraction with a commercial kit. The target regions of the ribosomal operon were amplified in polymerase chain reaction (PCR): ITS1 region with primers ITS1F-GC and ITS2 for fungal communities and 16S rRNA segment with primers F968-GC and R1401G for bacterial communities. PCR amplicons were separated using denaturing gradient gel electrophoresis (DGGE). DGGE profiles were analyzed in BioNumerics (©Applied Maths NV).
The DGGE profiles revealed differences in both fungal and bacterial communities among the different tree species and between the treatments. As for the different tree species, community profiles of western red cedar differed the most from the rest, especially the surface portion of the sample. The most notable change was observed in community profiles of ACQ treated spruce.
These results lead to new insights into the processes of wood biodegradation, and thus in potential protection treatments.
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Isolation and Screening of Highly Cellulolytic Trichoderma spp. from the Amazon Rainforest

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The Amazon rainforest is the largest Brazilian Biome. Its soil harbors a high microbial diversity that participate in the degradation of organic matter. The breaking of lignocellulolytic compounds by fungi in tropical forests becomes important for carbon cycling, and the fungus Trichoderma, is well know agent of decomposition of cellulose. Cellulose and hemicellulose components total some 60-70% of plant tissues and these have high importance because the sugars generated can serve as starting material for the production of second generation ethanol. This study aimed to isolate highly cellulolytic Trichoderma species from soil of the Amazon rainforest. The soil samples were collected in 13 sites, with three points in each in the Amazon state, covering a transect of, approximately, 600 Km. The plate screening assay with TSM medium with carboxy methyl cellulose was used in this investigation. Cellulolytic fungi were evaluated after 7 days for cellulolytic enzymes by staining with Congo red. The interaction of this stain with beta-D-glucans provides the basis for a sensitive assay for fungi possessing beta-(1-4), (1-3)-D-glucanohydrolase, beta-(1-4)-D-glucanohydrolase, and beta-(1-3-D-glucanohydrolase activities. A total of 820 strains of *Trichoderma* were isolated. All isolated strains were grouped based on cultural, biochemical and molecular approaches, and approximately, 300 strains were screened for cellulolytic activity. All strains were fermented in liquid medium supplemented with wheat bran and evaluated for total cellulase. *Trichoderma* isolates were grouped as high cellulolytic on the bases of cellulase activity compared with the highly cellulolytic strain *Trichoderma reesei* RUT C-30. Fifty-seven *Trichoderma* strains showed cellulolytic activity higher than the control, suggesting important role of this fungus in carbon cycling in the Amazon.
Keywords: Amazon Rainforest, Trichoderma, Cellulase.

Effect of wood extractives on wood-degrading microorganisms and importance of the ecological niche

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In forest ecosystems, wood degradation is an important process in biogeochemical cycles in term of nutrient and carbon cycles. Microorganisms such as saprotrophic fungi and bacteria are known to contribute in a significant manner to this process. However, during the first steps of wood degradation, low molecular weight compounds called extractives, are released, creating a chemical protective barrier against saprotrophic microorganisms.
The main goals of the study are (i) to evaluate the effects of wood extractives on the functional potential of wood-degrading bacteria isolated from the mycosphere of the white rot-fungi Phanerochaete chrysosporium and on the fungus itself, and (ii) to evaluate the role of the ecological niche by comparing the functional potential of soil and wood-degrading bacteria in presence of extractives.
Three-tree species, characteristic of European forests, were selected: oak (*Quercus petreae*), beech (*Fagus sylvaticus*) and Norway spruce (*Picea abies*). Heartwood sawdust extractions were carried out successively with four solvents of increasing polarities (dichloromethane, acetone, toluene/ethanol and water) using a soxhlet apparatus. For each extract, identification of the main families of chemical compounds and characterization of the main associated compounds were performed by gaz chromatography-mass spectroscopy (GC-MS).
Effect of wood extractives on bacteria was then evaluated with the characterization of their growth phases (lag phase, growth rate and generation time). Hydrophilicity and polarity of oak extractives were important parameters influencing negatively the microbial growth. For instance, inhibition of bacterial and fungal growth was positively correlated with the increasing hydrophilicity/polarity of oak extractives (ie. the highest inhibition was observed with extractives obtained with acetone, toluene/ethanol and water). Furthermore, it was demonstrated that the ecological niche is of importance regarding the functional potential of bacterial isolates in presence of oak extractives. Indeed, bacterial growth of soil isolates was further inhibited (time of lag phase is multiplied by 2-3 and the growth rate is sharply reduced) compared to bacterial growth of wood isolates.
Future directions of this work will be to identify the main compounds responsible for bacterial and fungal growth inhibition.

Turnover of Microbial Carbon in the Detritusphere

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In the frame of the DFG-Project (FOR 918) "Carbon flow in belowground food webs assessed by isotope tracers" we determined the carbon (C) flow and turnover of differently aged maize litter in bacteria and fungi of an arable soil. A microcosm experiment was set up for 60 days with ^{13}C labeled and unlabeled maize litter on top of soil cores. A reciprocal transplantation of the labeled litter on soil cores with unlabeled litter allowed us to follow the C flow into different components of the microbial food web during the decomposition process. A re-transplantation of unlabeled litter on top of soil cores that have been previously incubated with labeled litter was performed to quantify the ongoing depletion of the ^{13}C signal in different biotic and abiotic soil C pools. To determine the C turnover, soil cores were sampled destructively directly before litter transplantation and after 4, 8, 12 and 20 days, respectively.
The experiment allows an identification and quantification of different microbial groups feeding on substrate of different quality at three time points during litter decomposition. We expected specific microbial communities involved in maize litter decomposition at different time points. The litter transplantation during the first two weeks allowed us to follow the incorporation and turnover of easily available maize C by the microbial community, whereas during the progress of litter decomposition during the next weeks we followed the incorporation and turnover of more complex maize-derived C compounds by microorganisms.
We analyzed microbial CO_2 respiration, total soil organic carbon (SOC), extractable organic carbon (EOC), microbial biomass (Cmic) by chloroform fumigation extraction and the microbial community structure by phospholipid fatty acid (PLFA) analysis in the top three millimeters of the soil cores.
During the initial days of the experiment, we found a proportion of maize-derived C in the trapped CO_2 (up to 17%), which decreased with continuous decomposition of the litter (7% after 60 days). We detected a slight increase in the microbial biomass during our experiment. The maize-derived C in Cmic directly after the labelling phases was 4.4% (0-4 d), 1.2% (4-12 d) and 0.3% (28-36 d), respectively. The SOC pool showed a lower incorporation of maize-derived C of 0.16%, 0.1% and 0.04% at these respective sampling dates. The calculation of turnover rates in the different biotic and abiotic pools is still underway.

Organic N decomposition by fungal community under fertilized spruce forest

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Nitrogen fertilization in boreal and northern temperate forests diminishes the allocation of photosynthate C to the roots and reduces the growth of ectomycorrhizal fungi. Furthermore, the N addition in these ecosystems may lead to a reduced exploitation of the soil organic N by both plants and microorganism. In this study, we examined the organic material from mesh bags with maize compost placed in control and fertilized soils after colonization of fungal communities using infrared and near-edge X-ray absorption fine structure (NEXAFS) spectroscopies.
These mesh bags were filled with 70 g quartz sand particles and 2% (d.w.) of composted maize materials andplaced in a Norway spruce forest at Tönnersjöheden research park (southern Sweden). In this experimental site, three plots (30-40 m by 25 m) were amended with 200 kg N ha^{-1} in form of NH_4NO_3 and three were used as control. Six bags per plot were placed vertically along the soil profile to cover the humus layer and the upper part of the mineral soil. The mesh size of the bags (50 µm) allowed fungal hyphae to grow inside the bags but excluded the roots. The bags were collected after 17 months and the content was separated into one portion corresponding to the mineral layer and another one corresponding to the mineral soil part.
The diminution of total C and N content in the mesh bags from the mineral soil layer indicated a stronger modification of the organic material compared to the humus layer. The infrared spectroscopy showed that the dissolved organic matter of the mesh bags from the mineral layer in the fertilized soils had higher amidic compounds in comparison to that in the control soils, while the humus layer did not show significant differences between treatments. A further analysis of the mesh bag content from the mineral layer using NEXAFS spectroscopy showed that its organic material had been oxidized regardless of the N fertilization treatment and that a selective decrease of the peaks corresponding to amino acids and heterocyclic N had occurred in that from the control plots.
These results suggest that the decline of soil organic N species in the mesh bags from control plots was probably an effect of uptake and transport of N to the trees by the ectomycorrhizal fungi colonizing the bags. This effect diminishes when the forest is fertilized since the demand for N declines. Therefore, N fertilization may then be an important factor controlling decomposition of the organic N in forest soils.

Carbon dynamics in Amazonian podzols under climate change

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It has recently been shown that the C stocks in Amazonian podzols are very large. They are much larger than was previously thought, particularly in the Bh horizon, which has been estimated to contain in excess of 13Pg C for Amazonia alone. It is predicted that the changes in regional climate will result in a drier soil water regime which may affect the C dynamics in these soils that are usually saturated. In order to determine the vulnerability to change of the organic C contained in the Amazonian podzols, a series of incubation experiments were established in which the effects of a number of different factors on microbial decomposition were measured. The direct effect of drier soil water regimes was tested by incubating undisturbed cores from the Bh horizon at a range of matric potentials (saturation to wilting point). Contrary to what is usually found in soils, no significant difference in mineralisation was found among matric potentials, suggesting that other factors control microbial mineralisation of this organic C. The effect of nitrogen additions, of anaerobic conditions and of the addition labile C substrate were also tested on undisturbed cores of the Bh horizon of the podzols. Samples incubated under aerobic conditions produced 3 times more CO_2 than samples incubated under anaerobic conditions, whilst samples incubated under aerobic conditions with the addition of N mineralised 6.7 times more CO_2 than the anaerobic samples. The addition of labile C did not have a significant effect on C mineralisation, i.e. there was no priming effect. The combined addition of labile C and mineral N did not stimulate C mineralisation more than N additions alone. By extrapolating the differences obtained here to the whole of the Amazonian podzols, it is estimated that changes in conditions which result in an increase in O_2 and in N (i.e. changes in vegetation due to increases in dry periods with the establishment of a savanna for example) in the soil will cause the release of 0.14Pg C per year. This is equivalent to 0.2% of the CO_2-C released yearly by the world's soils.

Soil organic matter degradation by ectomycorrhizal fungi

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Ectomycorrhizal (ECM) fungi play a key role in nutrient cycling in boreal forests. Especially plant growth-limiting nutrients such as nitrogen (N) and phosphorus (P) are known to be taken up efficiently by ECM fungi and transferred to their plant hosts. However, most N and P in soils are not readily available for uptake by mycelia since they are mostly present in organic molecules such as amino acids, proteins and chitin or they are enclosed in, bound to or embedded in intact cell walls, lignin, etc. Hence, in order for ECM fungi and plants to gain access to these mineral nutrients, a broad spectrum of more or less complex organic molecules must be broken down. Recent studies suggest the involvement of Fenton-type reactions in the degradation mechanisms of soil organic matter (SOM) employed by ECM fungi. To investigate the chemical modifications introduced by ECM fungi to SOM in more detail, modifications of the main SOM components – lignin, cellulose, proteins (BSA), chitin and pectin – were studied with Fourier-Transform Infrared Spectroscopy (FTIR) in degradation experiments with the ECM fungi *Paxillus involutus* and *Suillus luteus*.

Fungal decomposition of fine roots in response to variable retention silviculture

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While variable retention silviculture has improved outcomes for many aboveground management objectives, only limited information is available regarding how this management practice influences belowground diversity and carbon (C) transformations. Many studies focus on the impacts of variable retention harvesting on litter decomposition dynamics, but few studies have identified how these alternative silvicultural systems can alter root-associated carbon. Greater attention is being directed toward understanding root-carbon dynamics as it is an under-studied carbon pool and is located within the soil where it is more likely to enter stable C-pools via fixation on clay particles or encasement in soil aggregates. The objective of this research is to examine the decomposition dynamics of fine roots in response to variable retention silviculture. The approach pairs a 2-year root litterbag mass-loss study with analysis of the structure and function of fungal communities degrading root litter in situ across three harvest treatments (clear-cut, aggregate and dispersed retention), an uncut control, and at two sites (near Campbell River, British Columbia). Fungal DNA extracted from root litter was sequenced across treatments after 45 days and 1 year of decomposition. Here, we present this fungal community data, root-litter mass-loss data after 1, 3, 6 months and 1 year of decomposition, and soil physiochemical data. This approach will assess how the decomposition rate of fine roots varies between harvest types, how fungal community structure differs between treatments and over time, and if any soil physiochemical variables can be identified that act as drivers of observed variation in mass-loss and community structure data.

Reciprocal Soil Transfer Experiments Improve the Understanding of Biological Regulation of Subsoil C-cycling

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While the habitat conditions influencing the abundance of microorganisms in topsoil are well investigated, these dynamics remain largely unexplored in deeper soil horizons. We started with investigations of both the abundance and the composition of the microbial communities in samples from top- and subsoil layers of a podzolic Cambisol from a European beech (*Fagus sylvatica* L.) forest in Lower Saxony, Germany. The samples were taken along three transects in a grid sampling design. Each transect consisted of 64 sampling points, eight vertical, from a depth of 10 cm to a depth of 185 cm, by eight horizontal, starting at the root zone of an individual beech tree, from zero to 315 cm. We found that the distribution of roots in subsoils was the main driver of microbial abundance and community composition. Consequently, we established two reciprocal soil transfer experiments using different amounts of ^{13}C labeled substrates (cellulose and roots) to evaluate the relative impact of surface/subsurface habitat conditions and resource availability on abundance, function and diversity of the soil microbial community. The duration of both experiments was 12 months. The microbial biomass of both top- and subsoil samples showed no significant changes while the activities of enzymes highly fluctuated depending on the treatment type. The decoupling of abundance and function of soil microorganisms might be due to N limitation restricting microbial growth and enhancement of microbial function under altered environmental conditions. Stable isotope methods (^{13}C biomass and ^{13}C PLFA) allowed us to follow the carbon flow from the added source into different microbial fractions. The incorporation of ^{13}C into the microbial biomass increased over the duration of the experiments depending on the amount of added ^{13}C labelled substrate. However, the different microbial groups showed significantly different incorporation rates. Bacterial groups showed a relatively low ^{13}C incorporation compared to the high incorporation into the fungal community, which shows the ability of fungi to explore resources in surrounding soil due to hyphal growth. Additionally, abundances of bacteria, fungi, archaea and seven taxa specific groups of bacteria were evaluated using qPCR to investigate changes of the community composition. These studies provided a deeper understanding of the influencing factors on abundance, function and composition of microbial communities in top- and subsoil habitats.

Recovering of soil protozoan trophic groups after a strong pulse of hydrocarbon contamination

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Soil food webs become simplified as a result of strong pulse of hydrocarbon contamination. However, these webs may recover based on hydrocarbon resisting bacteria, which in turn may benefit from root exudates. We aim to determine the time lapse of ciliates and flagellate trophic groups' reestablishment after a strong pulse of hydrocarbon contamination (50000 ppm light oil) in microcosm experiment planted with *Medicago sativa* (legume). Soil samples (40 g) were processed for protozoan examination after 0, 7, 14, 30, 60, 120 and 240 days after contamination (treatment 1), contamination plus seeding of *M sativa* (treatment 2), non-contaminated soil (control 1) and non-contaminated soil plus *M sativa* (control 2). Trophic groups were reduced to only bacterivorous immediately after contamination while other groups gradually recovered after 7 days. Trophic groups (bacterivorous, osmotrophs, autotrophs, protozoan eaters, fungivorous, algivorous) were totally recovered after 30 days in soils with *M sativa* and took 60 days in the control microcosm. All groups were represented throughout the 120 and 240 days in the contaminated microcosms (planted and non-planted). Species richness was higher in planted soils than in non-planted ones, while bacterivorous, autotrophs and fungivorous remained in the non-contaminated microcosms. Bacterivorous protozoans were always more numerous than any other group. Protozoan community share 100% likeliness in the contaminated microcosm (both planted and non-planted) and only reached 60% with the control soil. Protozoan communities recover relatively fast after a pulse of strong contamination and reached an alternate species composition in hydrocarbon contaminated soils no matter the presence of *M sativa*.

DNA-based determination of soil microbial biomass carbon under conditions of restricted applicability of substrate-induced respiration and fumigation-extraction

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Microbial biomass is a sensitive indicator of changes due to soil management. Improvement of methods for determination of microbial biomass still remains relevant, and these methods should be correctly applicable for the soil samples being in various state. This study was aimed to demonstrate the applicability of DNA-based determination of microbial biomass carbon under conditions when the common basic approaches, namely chloroform fumigation-extraction (CFE) and substrate-induced respiration (SIR), are restricted by certain soil properties, experimental designs or research needs, e.g. in frozen, alkaline or carbonaceous soils.

We compared microbial biomass carbon determined by CFE, SIR and by DNA approaches in the range of neutral and slightly alkaline Chernozem (pH from 6.5 to 7.6) and alkaline Calcisol (pH up to 9.1) of semi-arid climate. The samples of natural and agricultural ecosystems were taken throughout the soil profile from long-term static field experiments in the European part of Russia.

Extraction and subsequent quantification of dsDNA revealed a strong agreement with SIR and CFE when analyzing the microbial biomass content in soils with pH below 8. The conversion factors F(DNA) from dsDNA to SIR-Cmic (5.10) and CFE-Cmic (4.41) were obtained by testing a range of the soil samples down to 1.5 m depth and indicated a good reproducibility of DNA-based estimations. In alkaline soils with pH above 8, CO_2 retention due to alkaline pH and exchange with carbonates resulted in a strong underestimation of soil microbial biomass by SIR or even in the absence of any CO_2 emission, especially at low absolute values of microbial biomass in subsoil. Correction of CO_2 efflux by theoretical retention pH-dependent factors caused overestimation of SIR-biomass.

In alkaline conditions, DNA extraction proved to be a reliable alternative for microbial biomass determination. Moreover, the DNA-based approach can serve as an excellent alternative enabling correct estimation of microbial biomass in geographically widespread soils after their freezing.

The DNA-Cmic revealed that although the absolute values of microbial biomass in Chernozem were expectedly higher than in Calcisol, the Cmic:Corg ratio was greater in Calcisol versus Chernozem. Furthermore, DNA-based determination of Cmic and Cmic:Corg ratios revealed that agrogenic impact does not always lead to negative consequences for soil status and cannot be considered as a solely negative phenomenon.

Characterization of Fe^{3+} reductants secreted by the closely related ectomycorrhizal fungus *Paxillus involutus* and the saprotrophic fungus *Hydnomerulius pinastri* during Fenton-based decomposition of organic matter

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Ectomycorrhizal fungi are thought to have a key role in mobilizing organic nitrogen that is entrapped in soil organic matter (SOM). However, the extent and mechanisms by which ectomycorrhizal fungi decompose SOM remain unclear, considering that they have lost many genes encoding lignocellulose-degrading enzymes that are present in their saprotrophic ancestors. Nevertheless, using spectroscopic analysis and transcriptome profiling we have recently shown that in the presence of glucose, several species of ectomycorrhizal fungi convert lignocellulose components in SOM using oxidative mechanisms (Shah et al. New Phytologist, in Press). A comparison of closely related species within the Boletales clade showed that ectomycorrhizal fungi oxidized litter material as efficiently as brown-rot saprotrophs. Brown-rot fungi decompose lignin by means of an initial non-enzymatic step: attack by reactive oxygen species, including hydroxyl radicals generated by the Fenton reaction ($H_2O_2 + Fe^{2+} + H^+$? $H_2O + Fe^{3+} + \bullet OH$). In brown-rot fungi, the reactants for the Fenton reaction is generated by the secretion of extracellular metabolites that drives one-electron reductions of $Fe^{3+}$and O_2. Such a metabolite, involutin, was recently characterized from the ectomycorrhizal fungus *Paxillus involutus* (Shah et al. Applied and Environmental Microbiology, in press). *P. involutus* belongs to the Boletales clade which contains a number of ectomycorrhizal species that are nested within a paraphyletic assemblage of brown-rot decayers. The aim of this study was to compare the production of Fe^{3+}-reducing metabolites in *P. involutus* with that of the closely related brown-rot decayer *Hydnomerulius pinastri*. Activity-guided purification was performed to isolate the Fe^{3+}-reductants secreted during the decomposition of a maize compost extract. Analysis using HPLC showed that *P. involutus* secreted two fractions of Fe^{3+} reducing compounds of which one was identified by LC-MS as involutin. Under the same growth conditions, H. pinastri produced an array of other Fe^{3+} reducing metabolites. It remains to be determined whether the differences in the production of Fe^{3+}-reductants are associated with the different habitats (soil versus wood) and/or life strategies (symbiotic versus saprotrophic) of *P. involutus* and *H. pinastri*.

Bridging the priming effect into aquatic systems: Primary producer-C stimulates the fungal decomposition of submerged litter

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The quality of organic matter (OM) is highly variable and covers a large gradient of resistance to degradation. Recalcitrant OM can be 'activated' and thus involved in carbon (C) and nutrient cycling in ecosystems through the priming effect, which is the increased mineralization of recalcitrant organic matter triggered by inputs of labile OM. As such, the effects of labile C on the decomposition of OM is of considerable interest for soil microbial ecology, especially in the rhizosphere where fluxes of labile C are high. However, less is known about the importantance of labile C input for the turnover of OM in other systems. A situation similar to the rhizosphere can be found, e.g., on submerged plant material in the presence labile C input by photosynthetic algae. A central challenge for research on the priming effect is a relevant delivery of labile C. Most studies to date have used pulse additions of single substrates at high concentrations. This may not represent a natural environment well. To achieve an assessment of the priming effect it is important to simulate a realistic delivery of labile organic matter, capturing a continuous but dynamic delivery of low concentrations.
We used pond water microbial communities where plant litter additions were submerged. We monitored the successional dynamics of fungal (acetate incorporation into ergosterol) and bacterial growth (leucine / thymidine incorporation), primary production (PP) activity, and respiration on litter under dark and light conditions. We also mimicked the delivery of PP labile C by continously adding ^{13}C-glucose at an identical rate using perilstatic pumps in dark systems, thus tracing labile C into respired CO_2 and microbial groups (SIP-PLFA). PAR light was used to minimally impact photodegradation of OM. We observed an increased fungal production and abundance in light treatments. The fungal growth response coincided with an increase in algal primary productivity occurring at day 7. Dark treatments showed a low fungal growth and no primary production. Bacterial production increased rapidly in the first days but decreased with no differences between light and dark systems. Glucose additions induced comparable effects to the light systems with active PP, and enabled the partitioning of sources for the respired $^{13}CO_2$. We conclude that primary production can stimulate fungal growth and that the presence of labile C consequently can 'prime' the decomposition of litter.

Influences of carbon substrates and nitrogen availability on microbial-mediated cellulose degradation in an Austrian beech forest soil

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The microbial degradation of plant biomass represents an important step within the terrestrial carbon (C) cycle. Cellulose is a key component of plant material, and thus one of the major constituents of this soil C-pool. Microorganisms (bacteria, archaea and fungi) are mediating the decomposition of this most abundant polymer on Earth, but their exact contributions and ecological niches are still not fully understood. Microbial-mediated cellulose degradation was investigated in an Austrian beech forest soil in a time course microcosm experiment. We hypothesized that by varying certain soil properties that can limit cellulose degradation, we will uncover the active cellulose-responsive bacterial and fungal communities and their associated ecological niche(s) in this process. Destructive soil microcosms amended with ^{13}C-cellulose were used to identify the bacterial and fungal response to different types of background C and nitrogen (N) amendments over 25 days. Cellulose utilization was monitored by measuring respiration ($^{13}CO_2$) and cellulolytic enzymatic potential, in combination with characterizing the cellulose-responsive microbial community using ^{13}C-phospholipid-fatty-acid-stable-isotope-probing (PLFA-SIP) and ^{13}C-DNA-SIP. General respiration activity for C amended microcosms, as measured by $^{12}CO_2$, exhibited the highest rates of activity, yet the N amended microcosms exhibited the highest rates of cellulose degradation activity (measured by $^{13}CO_2$ and cellobiohydrolase activity). We further elucidated diverging microbial responses to the various treatments through PLFA-SIP. The proportion of fungal and bacterial PLFAs increased in the N amended and C amended treatment, respectively, suggesting differential niches in cellulose degradation of these different groups. More detailed analysis of cellulose-responsive bacteria via T-RFLP analysis of 16S rRNA genes depicted a distinct bacterial community composition across the C and N amended treatments and time suggesting niche differentiation even within the bacteria. To identify these cellulose-responsive communities (bacteria and fungi), high throughput sequencing of the ^{13}C enriched DNA is currently underway. Thereby, we will be able to elucidate the active microbial participants in cellulose degradation and to identify their associated ecological niches, which will allow us to better understand the participants in the degradation of cellulose – an important step in the terrestrial C cycle.

Fungal biodiversity of wood decomposing species in national nature reservation of Salajka

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The aims of this study were detection and determination of fungal species presented in dead wood material based on the influence of duration of decay and the tree species in a national nature reservation of Salajka on the east of Czech republic. During autumn of 2014, 120 logs were drilled, at the bottom and at the top. Always two drilling per position were then put together, so finally we acquired 240 samples - 2 per a log - which were further processed. The samples were grounded up and stored in -80°C until the isolation of DNA. After the isolation, the samples were kept in -20°C until the amplification of 18S rRNA, using the primer ITS1 a ITS4. We sequences the samples and statistically analyzed them.

Litter decomposition in mangroves: the role of microbes revealed by DNA and mRNA sequencing

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Mangroves are costal ecosystems known for its important role as sink and source of organic matter. This ecosystem covers most of the tropical and subtropical shores. A major portion of the organic matter that is produced by the mangrove vegetation is exported to nearby marine ecosystems. Therefore, this study was designed to shed light over the process of litter decomposition by using high throughput sequencing of the mRNA and DNA present in decaying mangrove leaves. With this purpose, litter bags contatining leaves of *Laguncularia racemosa*, *Avicennia schaueriana* and *Rhizophora mangle* were left on the mangrove sediment for 60 days. This experiment was conducted in three different mangroves in different locations. These material was had its total DNA and mRNA extracted and used to construct libraries that were sequenced in an ion torrent PGM. Sequences were annotated by MG-RAST, CAZY and Crass. The community patterns were very similar between plants; however, there were significant differences between locations. There were some important differences between the groups observed for in the DNA and mRNA libraries. The major phylogenetic groups observed were Gamma and Deltaproteobacteria while the functions were clustering-based subsystems, carbohydrate, amino acid and derivatives, protein, miscellaneous metabolism. The search for carbohydrate metabolism genes using CAZY found genes related to plant organic matter decomposition (GH26, GH17, AA3 and GH43). The major difference was a switch between Gamma and Deltaproteobacteria as dominant classes between mRNA and DNA (respectively). Another striking difference was that the DNA libraries were much more similar, despite the geographical distance than the mRNA. Hits findings highlight the importance of environmental signals to the expression of genes.

Using teabags to estimate decomposition rates across primary and secondary tropical forests, and investigating the functional role of termites

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Decomposition is a vital ecosystem process, forming a major pathway in the cycling of carbon and nutrients. This process is biotically regulated by organisms within the decomposer community. In tropical forest ecosystems, termites serve as the dominant invertebrate decomposer of plant litter.

Widespread land-use changes in the tropics have led to the increasing prevalence of tropical secondary forests, which support a reduced and/or altered community of decomposers, including termites. While many studies have highlighted the importance of biodiversity in maintaining ecosystem processes, there remain uncertainties in the explicit relationships and mechanisms linking invertebrate decomposers' diversity and decomposition rates.

Previously, studies on decomposition typically employed the litterbag method, which is time-consuming and more importantly – plagued with methodological inconsistencies. In this study, we adopted a recently proposed method (Tea Bag Index) to obtain reliable and hence comparable rates of decomposition. We had two main research objectives. Firstly, we investigated the influence of a termite diversity gradient across primary and secondary forests on decomposition rates. Secondly, we validated the assumptions of the Tea Bag Index and assessed its applicability in tropical forest ecosystems.

Our results showed that old-growth forest sites supported the slowest rate of decomposition. This is contrary to the general consensus of a positive biodiversity-function relationship. Although the secondary forest sites harboured a lower richness of termite species, the altered community assemblage of termites appeared to have accelerated decomposition rates beyond those observed in primary forests.

Furthermore, our results confirmed that in addition to microbial decomposers, termites are functionally important in the decomposition process too. By using invertebrate exclusion techniques, we showed that the Tea Bag Index and its underlying assumptions are invalid in tropical forests where termites are abundant and speciose.

Lastly, we suggest making modifications to the Tea Bag Index to retain its global validity. This will permit the collection of decomposition data at finer spatial resolutions, allowing the atmosphere-biosphere carbon balance to be modelled with greater confidence. It is hoped that this study will serve to emphasise the far-reaching consequences of bottom-up changes in the decomposer community on the global carbon cycle.

Linking deadwood age with inhabiting bacterial community

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The decomposition of deadwood substantially contributes to the carbon cycle and therefore is one of the key processes in temperate forests. This process is driven by saprotrophic organisms such as fungi and bacteria. While the role of fungi in deadwood decomposition was repeatedly addressed, there are just a few surveys of deadwood-associated bacteria. Dead trees at dissimilar level of decay can harbour specific community of bacteria adapted to exploitation of woody substrate in the neighborhood of fungi. Our goal was to describe the composition of bacterial community in the different phases of decomposition of *P. abies*, *A. alba* and *F. sylvatica* logs in the Zofin Natural Reserve, Czech Republic. We used comprehensive tree and log database for linking community with the age of logs. Samples were obtained by drilling and the composition of bacterial community was characterized by 16S rRNA sequencing on the Illumina MiSeq platform. Further, hundreds of bacterial isolates were cultivated with intention to genomic, enzymatic and substrate specificity characterization of some abundant and elusive taxa. Members of the classes ?-, ?-, and ?-Proteobacteria and the phyla Actinobacteria and Acidobacteria were dominant throughout decomposition. The genera *Mucilaginibacter*, *Burkholderia* and *Steroidobacter* which were previously described as cellulolytic were detected as abundant. The genus *Pseudomonas* described as lignin-modifying was observed in high abundances. Presented study shows that communities in older logs are structurally different and less diversified than communities in fresh deadwood, possessing adaptations for long term-survival in decomposing substrate. Moreover higher abundance of the phylum Acidobacteria together with successfully cultivated isolates from this phylum promise new insight into the role of bacteria in the process of deadwood decomposition.

Sequence processing fast and easy: SEED a GUI based user friendly sequence editor and pipeline for high-throughput amplicon processing

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Although there are many well established pipelines for amplicon data analyses, most of them are harder to comprehend and handle by biologists without necessary command line skills and background in bioinformatics. The SEED (http://www.biomed.cas.cz/mbu/lbwrf/seed/) is a free-to-use GUI-based sequence editor and pipeline for Windows platforms providing access to internal functions as well as those performed by external software that is installed for full functionality (1). SEED was created to provide an intuitive interface for fast bioinformatic analysis of PCR amplicons on desktop computers according to the suggested workflow. The most recent 64-bit version allows comfortable work with up to 8 million sequences (~4 GB of data) on a standard personal computer with 8 GB RAM. SEED is especially suitable for sequential analysis of the sequences of PCR amplicons obtained by Illumina or 454-pyrosequencing, such as sequences of fungal ITS regions, bacterial 16S rDNA or other target genes. The program has a wide array of functions including editing of sequences and their titles, sorting, quality trimming, pair-end joining, grouping of sequences based on sequence motifs or sequence titles, batch processing of sequence groups, denoising, chimera removal, ITS extraction, sequence alignments and clustering, OTU table construction, construction of consensus sequences, creation of local databases for BLAST and searching either them or the whole NCBI, retrieval of taxonomical classification from the NCBI, calculation of diversity parameters and many more.

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Agro-ecosystem type and soil aggregate size impact soil carbon dynamics

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Many microbes possess the ability to utilize cellulose, the most abundant polysaccharide on Earth, thereby making crucial contributions to global carbon dynamics. However, the link between specific soil habitat characteristics, microbial community composition, and cellulose utilization remains poorly understood, hampering our ability to make accurate predictions of soil carbon dynamics in future climate change scenarios. To elucidate the link between soil structure, specifically soil aggregate size, agro-ecosystem, and microbial cellulose utilization under different climate conditions, we performed a microcosm study using two soil aggregate size fractions from two different agro-ecosystems, continuous corn and fertilized prairie, and incubated them with and without cellulose addition at different temperatures and soil moistures. We tracked soil respiration over time and determined enzyme activity profiles, and microbial biomass carbon and nitrogen at the end of the 10 day incubation study.
Results show that cellulose addition resulted in 2.2 and 6 times higher cumulative respiration in the fertilized prairie and corn ecosystems respectively compared to control treatments without cellulose. However, there was little effect on enzyme production and biomass, suggesting that cellulose is utilized
to increases the metabolic activity of the soil microbial communities rather than building of biomass. Small macro-aggregates (< 250 µm - 1 mm) produced more CO_2 than large macro-aggregates (> 2 mm; $P < 0.001$) and built more biomass ($P = 0.002$). This suggests that small aggregates, that compose only a small proportion of soil mass, are of great relative importance to soil processes. Interestingly, while most enzyme were unaffected by moisture, ?-glucosidase and cellobiohydrolase, which are both involved in the decomposition of cellulose, showed higher concentrations in dry than in wet treatments. Higher temperatures led to increased respiration but did not affect enzyme activity or biomass.
The ecosystem with corn as cropping system produced significantly more CO_2 when cellulose was added than an ecosystem with a diverse plant community, indicating that diversity of cover crops is crucial to the functioning of carbon cycling soil microbial communities. Overall, our results suggest that agro-ecosystems are of great importance to microbial carbon dynamics in soil.

Microbial immobilization and incorporation into DNA of inorganic ^{33}P-labelled phosphorus

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Microbial biomass P (Pmic) represents one of the most labile soil P pools providing fast processes of P immobilization-mineralization. The sustainability of P retention in Pmic depends on the P partitioning between cell compounds. Our research was aimed to estimate immobilization of readily available P by soil microorganisms and the percentage of P incorporation to one of the principle cell components, DNA.

Pmic and input to DNA was determined in four soil types: Podzol (Corg 3.3%, pHH_2O 3.5), Phaeozem (Corg 1.4%, pHH_2O 5.6), Chernozem (Corg 3.4%, pHH_2O 6.9), and Calcisol (Corg 1.9%, pHH_2O 8.3). Two treatments were used: soil labeled with a ^{33}P spike (0.1 mkg P g^{-1}); glucose amended soil (4000 mkg C g^{-1}) labeled with ^{33}P and fertilized with K_2HPO_4 (5 mkg P g^{-1}). Soils were incubated at 22°C and 70% WHC during 3 days and then analyzed for ^{33}Pmic and ^{33}P-DNA pools. Total Pmic was determined as well.

DNA was extracted from soil samples using standard MP Bio kit. Pmic was determined by direct fumigation and anion exchange membrane techniques. P concentration in extracts was determined by the malachite green colorimetric procedure, ^{33}P enrichment was measured by radioactivity. Total Pmic was calculated using conversion factors kP. The latter was individually determined in a series of experiments with ^{33}P labeling and measuring chloroform released ^{33}P recovery with correction for ^{33}P sorption and ^{31}P – ^{33}P isotopic exchange.

Conversion factors kPs were found to be treatment- and soil dependent varying from 0.19 to 0.40, with general trend for kps values in glucose amended soils to be higher than in glucose untreated. Total Pmic, labeled ^{33}Pmic and labeled ^{33}P-DNA pools in glucose amended treatment were also higher than those in glucose untreated soils. ^{33}P recovery in DNA varied from 8.8 % (Chernozem, glucose untreated) to 37.2 % (Podzol, glucose amended) of ^{33}P added. When expressed as a ^{33}P-DNA share of the labeled ^{33}Pmic, four soils were split into two groups, with relatively low (26 – 60%, Phaeozem and Calcisol, Corg < 2%) and extremely high (83-85%, Chernozem and Podzol, Corg > 3%) ^{33}P incorporation into DNA.

Thus, P immobilization by soil microbial community and P incorporation into DNA was stimulated by labile C substrate (glucose). The share of newly-immobilized labeled P in DNA was higher in SOM-enriched soils than in soils with low SOM content.

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Correlation of lignocellulolytic genes expression and their activity in ME fungal cultures

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Nowadays, with the advent ofgenomics and transcriptomics, we are trying to elucidate elusive connection between coded and expressed genes and their active protein products. The aim of our study was to identify the relationship between expression of lignocellulolytic mRNAs and activity of secreted enzymes in pure fungal cultures.
We have grown 28 different species of fungi on 50ml of liquid malt extract medium with addition of 0.1g of dried and milled straw to induce production of lignocellulolytic genes. 21 of these fungi were Basidiomycota and 7 were Ascomycota. Liquid medium was sampled each week from total 4 weeks, for enzyme activity assays. Total RNA was isolated from fungal mycelia as soon as enough biomass was available and enzymes were detected (2nd and 3rd week). New protocol was established for rRNA ribosomal removal after RNA fragmentation and RNA index ligation. 6 RNA fungal species were pooled together prior rRNA removal and library preparation. In total we had 5 rRNA depleted libraries for HiSeq sequencing.

Optimizing the toolbox to investigate free-living diazotrophs in soil: from bulk measurements to single-cell analysis.

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Biological nitrogen fixation is the only known metabolic process that converts atmospheric N_2 gas into biologically available form. It is carried out by a single guild of microorganisms – the diazotrophs. Diazotrophs are found in various bacterial and archaeal taxa and can be free-living or in symbiosis with plants. While the N_2 fixation rate of symbiotic diazotrophs is much higher than that of free-living diazotrophs, the latter are of great importance on a global scale due to their large numbers and wide range of distribution. However, knowledge about the identity and ecophysiology of free-living diazotrophs is still limited, partially due to the difficulty to study this guild using cultivation methods. Thus, cultivation independent methods have been proven to be essential for studying this important guild. It is our goal to optimize and develop tools for the identification and detailed investigation of active, free-living diazotrophs using $^{15}N_2$ stable-isotope labelling, advanced molecular tools and single-cell analysis techniques. Soil samples from an Austrian beech forest and a grassland were incubated in the presence of $^{15}N_2$. To reliably detect N_2 fixation activity at the highest sensitivity we have analysed the incorporation of ^{15}N into DNA, RNA and proteins and compared their ^{15}N enrichment to the bulk soil. We observed that tracing the accumulation of ^{15}N into biomolecules, particularly RNA, allows detecting nitrogen fixation activity sooner than when bulk soil is analysed, and as quickly as within one day of incubation. Furthermore, to identify the active diazotrophic community in soil samples we have developed an RNA-stable isotope probing assay (^{15}N-RNA-SIP) and compared this assay to the established ^{15}N-DNA-SIP assay. Besides being potentially less biased by differences in G+C content, ^{15}N- RNA-SIP facilitates a more rapid identification of diazotrophs, even if cells have not divided, which was in our experiments within 7 days. The activity of diazotrophs identified by ^{15}N-RNA or ^{15}N-DNA-SIP can then be verified using the FISH-NanoSIMS approach. In addition, we are currently testing the applicability of Raman microspectroscopy to detect single cells enriched in ^{15}N, as it was previously shown to be possible for ^{13}C and deuterium. In summary, the presented toolset allows the identification and investigation of active free-living diazotrophs in a highly sensitive manner in a variety of environments, from the bulk to the single-cell level.

Effect of phenanthrene on the release of mobile organic matter and the bacterial community structure in soil

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The water flow through soil pores transports mobile organic matter (MOM) comprising dissolved and particulate organic matter and microorganisms from the organic-rich topsoil to the subsoil. MOM is an important carrier of nutrients and contaminants governing their fate and mobility in soil. During transport, MOM is assumed to undergo various interactions with solid soil surfaces. We hypothesized that the presence of an additional carbon source (model compound phenanthrene) affects the composition and release of MOM, consequently altering the bacterial community and further soil parameters in the soil profile. We used a two-layer column study under water-unsaturated flow conditions. Columns (10 x 12 cm) were filled with Luvisol either spiked with phenanthrene in the upper 2 cm (0.2 mg g^{-1}) or left unspiked (control). Columns were irrigated with artificial rain water (0.5 ml min^{-1}) with alternating flow/no flow periods for overall 136 days. After the irrigation, columns were sliced into several layers for depth-differentiated analysis. The bacterial community structure was studied by denaturing gradient gel electrophoresis (DGGE) and pyrosequencing of 16S rRNA genes amplified from total community DNA which was extracted from effluent samples and three soil slices. Furthermore, the presence of genes involved in the degradation of phenanthrene and physico-chemical parameters (e.g. pH, TOC/DOC, anions, organic acids) were determined. A high variation in the mobile bacterial community structure was found by DGGE analysis of effluent samples. Release of MOM was controlled by physical non-equilibrium, and only small effects of phenanthrene were observed on effluents. The bacterial community structure in the soil profile of unspiked columns revealed only a few depth-depending variations, while pronounced shifts were observed in the bacterial community composition and abundance of catabolic genes due to phenanthrene spiking. Strongest positive responders to phenanthrene were affiliated to the genus *Geothrix* (Acidobacteria).
This study contributes to the understanding of release and transport of MOM including bacterial cells in soil. An effect of MOM on the establishment of soil interfaces and the microbiota is suggested. Therefore, column experiments with pristine model materials (artificial soils) were conducted which are presently under investigation.

Unexpected stimulation of soil methane uptake by bio-based residue application: An emerging property of agricultural soils offsetting greenhouse gas balance

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Intensification of agriculture to meet the global food, feed, and bioenergy demand entail increasing re-investment of carbon compounds (residues) into agro-systems to prevent decline of soil quality and fertility. However, agricultural intensification decreases soil methane uptake, reducing and even causing the loss of the methane sink function. In contrast to wetland agricultural soils (rice paddies), the methanotrophic potential in well-aerated agricultural soils have received little attention, presumably due to the anticipated low or negligible methane uptake capacity in these soils. Consequently, a detailed study verifying or refuting this assumption is still lacking. Exemplifying a typical agricultural practice, we determined the impact of bio-based residue application on soil methane flux, and determined the methanotrophic potential, including a qualitative (diagnostic microarray) and quantitative (group-specific qPCR assays) analysis of the methanotrophic community after residue amendments over two months. Unexpectedly, after amendments with specific residues we detected a significant transient stimulation of methane uptake confirmed by both the methane flux measurements and methane oxidation assay. This stimulation was apparently a result of induced cell-specific activity, rather than growth of the methanotroph population. Although transient, the heightened methane uptake offsets up to 16% of total gaseous CO_2 emitted during the incubation. The methanotrophic community, predominantly comprised of Methylosinus may facilitate methane oxidation in the agricultural soils. While agricultural soils are generally regarded as a net methane source or a relatively weak methane sink, our results show that methane oxidation rate can be stimulated, leading to higher soil methane uptake. Hence, even if agriculture exerts an adverse impact on soil methane uptake, implementing carefully designed management strategies (e.g. repeated application of specific residues) may compensate for the loss of the methane sink function following land-use change.

Land-use intensity and physico-chemical soil properties have distinct effects on microbial communities and enzyme activities of grassland soils

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Nutrient cycling in soil heavily depends on the present microorganisms and enzyme activities. In our study we assess if and how microbial communities and soil enzyme activities are influenced by the land-use intensity, i.e. grazing, mowing and fertilization intensity, of grasslands. 150 sites in three regions of Germany, differing in climate and soil characteristics, were selected: Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb. Bulk soil samples, which were taken from 0–10 cm depth in May 2011 and 2014, were analysed for microbial biomass (CFE), microbial community composition (PLFA patterns) and soil enzyme activities involved in carbon, nitrogen and phosphorous cycling. These biological soil properties were related to land-use intensity and physico-chemical site characteristics (bulk density, soil texture, soil type, pH, water retention curves, and C and N content).
First results of the Schwäbische Alb showed a strong negative correlation of soil bulk density with microbial biomass and the organic carbon content. Land-use intensity was positively correlated with urease and xylosidase activity as well as with gram positive bacteria. While gram positive bacteria were positively correlated with soil nitrate, fungi were not affected.
The results showed a distinct influence of land-use intensity and physico-chemical soil characteristics on the investigated biological soil properties. Linking the biological and physico-chemical soil properties revealed promising insights into nutrient cycling in grassland soils.

Shifts of C and N isotopes in fruiting bodies of fungi after 12 years of irrigation of a semi-arid pine forest

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A mature semi-arid Scots pine forest in southern Switzerland was irrigated during a 12-year period to reduce the ongoing drought stress on the trees. Irrigation of the forest site with doubling the precipitation amount led to a depletion of the ^{13}C-isotope in the assimilated C, and ^{13}C values in fine roots shifted from about -26‰ in the controls to about -27‰ in the irrigated plots. Similarly, ^{13}C values shifted in the litter layer and in the uppermost soil horizons. The corresponding ^{15}N values, however, showed a contrary trend with more negative values in the control plots. In order to investigate the functioning of ectomycorrhizal and saprotrophic fungi in C and N cycling the ^{13}C- and ^{15}N-isotopes were measured in their fruiting bodies. Collected fungal species such as *Cantharellus*, *Chroogomphus*, *Hebeloma*, *Lactarius*, *Russula*, and *Suillus* have an ectomycorrhizal lifestyle, whereas *Agaricus* and *Clitocybe* have a saprophytic lifestyle. The lifestyle of *Entoloma* is not fully elucidated. ^{13}C and ^{15}N values of these fruiting bodies will be presented from the control and the irrigated plots and shifts due to the treatment and the fungal lifestyles will be compared with other forest manipulation studies. Combining natural abundance with ecosystem manipulation might be a powerful technique to examine C and N sources of different fungi.

Ammonia oxidizers in a non-nitrifying Brazilian savannah soil

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Nitrate is one of the main forms of nitrogen taken up by plants and, when not incorporated, is easily leached, causing groundwater pollution and N loss. Brazilian savannah soils have low to undetectable nitrification rates, puzzling researchers for decades, and making it a unique model biome to study determinants of ammonia oxidation. Recently we linked the absence of nitrification in an undisturbed grassland savannah soil to a significantly lower abundance of archaeal (AOA) and bacterial (AOB) ammonia oxidizers than in an adjacent nitrifying soybean soil. We hypothesised that this was due to the lower pH in the grassland soil. We also hypothesised that the low water potential found in savannah soils during the dry season was limiting nitrification and that simulation of rain by increasing soil moisture would allow higher microbial activity, i.e. mineralization and subsequent ammonia oxidation. Finally, it was proposed that inhibitors in these soils, potentially produced by plants, could hamper ammonia oxidation. To test these hypotheses, we manipulated moisture and pH in microcosms containing grassland savannah soil. Nitrification inhibition was tested with slurries assembled with a mixture of savannah grassland and Craibstone soil, a Scottish agricultural soil whose nitrification activity has been well studied. Very little nitrate accumulation was observed in the savannah grassland microcosms with either increasing moisture or pH, despite high ammonia concentration. In the grassland slurries, AOA *amoA* transcripts were only detected after 14 and 21 days and not in all replicates. As expected at the acidic pH, AOA were 1 to 2 log units more abundant than AOB and no AOB transcripts were detected. In contrast, nitrification was not inhibited in the mixed soil slurries, final nitrate content being proportional to initial Craibstone/savannah soil ratios, indicating only a dilution of the ammonia oxidiser community, and not inhibition. In addition, DGGE profiles of the active community were similar in the mixed and nitrifying soils. Together, these results suggest that neither water availability, ammonia availability, low pH nor inhibition by soil compounds constrained nitrification in Brazilian savannah soils. This distinctive pattern, i.e. the absence of nitrification despite the presence of AOA and AOB, might be associated with a particular community, specialized in high N immobilization in organic matter rather than in N loss through nitrification.

Different agricultural practices drive aerobic and anaerobic ammonia oxidisers niche segregation in a temperate paddy soil

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Rice is a non limited ammonium-NH_{4+} agrosystem due to a high degree of mineralization process and fertiliser addition. Nevertheless, a low nitrogen (N) fertilizer use efficiency is acknowledged due to high N losses through aerobic-anaerobic ammonia oxidation. Therefore, it is crucial to comprehend the microbial control of these processes as a function of different crop residue and water management practices. The aim of this study was to investigate aerobic and anaerobic ammonia oxidisers niche specialization induced by fertilizer addition and mineralization products as NH_{4+} and DOC.
The research was carried out in four plots within an experimental rice platform (NW Italy) having different water and straw management practices as follows: F-NS: conventional flooding, straw removed; F-S-SPR: conventional flooding, straw incorporated in spring; F-S-AUT: conventional flooding, straw incorporated in autumn; DRY-S-SPR: dry seeding and 1 month delayed flooding and straw incorporated in spring. Soils samples were collected during May-June 2014 in correspondence of alternating dry-flooded periods for N fertilizer application. Molecular and chemical analyses were adopted to study the microbial communities dynamics in terms of potential functional abundance.
The abundance of aerobic ammonia oxidisers was not influenced by water management, suggesting nitrification as proxy for complete denitrification. Ammonia-oxidizing archaea (AOA) outcompeted the bacterial counterparts (AOB) in all treatments. The drying-rewetting cycles had a negative influence on AOA abundance which decreased over time. Important variation in Anammox bacteria (AMX) abundance between treatments was shown. Characteristic treatment interactions and cooperation were shown among AOA, AOB and AMX communities. However, the different relationship between DNA abundance and extractable and dissolved organic carbon (DOC) and NH_{4+} highlighted the possibility of AOA, AOB and AMX niche differentiation, not only driven by specific responses to NH_{4+} concentration, but also to organic C sources.
The results suggest an unexpected great influence of organic C on aerobic and anaerobic ammonia oxidation communities dynamics, with important implications on their potential functionality. This study is a step forward in understanding the overall microbial dynamics in mitigating N losses from rice fields.

Impact of bioavailable phosphorus on plant and soil microbial communities in grassland under restoration management

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Semi-natural grasslands are often hotspots of biodiversity, but their extent decreases steadily in Western Europe[1]. The European Habitats Directive therefore aims at their protection and restoration[2]. Several western European countries seek to restore semi-natural grasslands through conversion of agricultural land. However, development of species-rich grassland communities on abandoned agricultural land faces several major bottlenecks. Besides the potential unavailability of seeds of target plant species[3], the high nutrient levels and disturbed soil organism communities may further hamper restoration. First, due to years of intensive fertilizer application, soil nutrients stocks have built up to unprecedented levels. Among the nutrients, phosphorus (P) is particularly persistent[4]. High concentrations of bioavailable P have been shown to be negatively associated with plant species diversity[5,6] and occurrence of rare species[6]. A second major bottleneck for ecosystem restoration on abandoned agricultural land is the general absence of a typical community of soil organisms. Invertebrate soil fauna and symbiotic and pathogenic soil microbes have the potential to influence the composition of plant communities, either through changing the availability of resources or by direct interfering with plant growth[7,8].
This lecture aims to find out if and how P bioavailability (both inorganic and organic P) influences the soil microbial and plant communities in grassland ecosystems under restoration management. Until recently, the microbial community has been largely disregarded as possible driver of plant community dynamics[9,10], and information on how soil resources affect the microbial influences on plant communities is scarce[10,11]. We focus on moderately dry Nardus grasslands (Nardo-Galion) situated on sandy soils.
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How does long-term nitrogen input influence stoichiometric relationships between soil microbes and their resources?

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The below-ground part of terrestrial carbon (C), nitrogen (N) and phosphorus (P) cycles are controlled by soil microbial communities. However, microbes are largely stable (i.e. homeostatic) in terms of elemental composition while the C:N:P stoichiometry of their resources can vary widely. This disparity has been termed “elemental imbalance”.
In addition to natural variation, resource stoichiometry can also be influenced by anthropogenic perturbations of elemental cycles. Specifically, increased N availability as a result of atmospheric N deposition might reduce C:N and P:N ratios of resources which in turn might reduce corresponding elemental imbalances for soil microbes.
Soil microbes might overcome stoichiometric limitations of growth employing several mechanisms, including (i) non-homeostatic behavior of microbes on a cell and/or on a community level, (ii) the release of specific enzymes targeting C,N or P, respectively, and (iii) excretion of elements in excess, a process that involves adjustment of element use efficiencies.
Here, we make use of two long-term N addition experiments in Denmark and Switzerland to specifically test how soil microbial communities cope with elemental imbalances introduced by N inputs. Both forest stands are dominated by Picea abies that have developed on different soils (Podzol and Gleysol, respectively). The sites have been receiving N inputs receiving 30-35 kg N ha^{-1} y^{-1} for >20 years on top of 12-25 kg N ha^{-1} y^{-1} of background N deposition. In 2014, we took samples from organic and mineral soil horizons and analyzed concentrations of C,N, and P in soil and microbial biomass as well as activities of five hydrolytic enzymes targeting C,N and P-containing substrates.
First results show, that soil microbial communities are largely homeostatic with regard to C:N, but adjust corresponding enzyme activities to N input: At the nutrient poor site in Denmark, microbes increased investment in N-acquisition relative to C (i.e. lowered C:Nenz ratio), whereas we observe increased C:Nenz ratios in the upper organic horizons in Switzerland. We hypothesize that this divergent response is partly explained by the fact, that some N-acquiring enzymes also release C from organic substrates and that an increase in these activities might additionally indicate a shift in C-source.

The effect of temperature on the carbon isotope value of acetate in Philippine rice field soil

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Acetate is an important intermediate in the anaerobic degradation of organic matter. It is not only produced from fermentation but also from the reduction of CO_2 via the acetyl-CoA pathway (acetogenesis). Temperature is a major driving force in many environmental systems. To investigate the effect of temperature on acetate production in rice field soil, we incubated Philippine rice field soil at 15, 30 and 50°C under H_2/CO_2 or under N_2 with bromoethanesulfonate (BES) as inhibiter of methanogenesis and KCN as inhibitor of acetogenesis, and followed the carbon isotope signatures of CH_4, CO_2 and acetate by mass spectrometry. Compared with -15.9‰?-25.4‰ under N_2, incubations under elevated H_2/CO_2 yielded ^{13}C-depleted acetate of -68.0‰ ?-24.0‰, indicating that H_2-dependent acetogenesis dominated acetate production. Under N_2, however, carbon isotopic fractionation of acetate could only be observed at 15°C in incubations with KCN but not at the other temperatures (30 and 50°C). The ^{13}C values of acetate under these conditions were similar to those of soil organic matter (-23.5‰) indicating that acetate was mainly produced from fermentation.

Phosphate solubilizing microorganisms isolated from root and rhizosphere soil of ericaceous shrubs in the north of Morocco.

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Many soil microorganisms are able to transform insoluble forms of phosphorus to an accessible soluble form, contributing to plant nutrition as plant growth-promoting microorganisms (PGPM).
The objective of this work was to isolate, screen and evaluate the phosphate solubilization activity first, of fungi in the root, and second actinobacteria in the soil of ericaceous shrubs to select potential microbial inoculants.
Soil samples (pH=5,46, available P 0,260 mg/g, Total N 2,5ppm organic matter 2,5%) were collected from the rhizosphere of ericaceous soil in the area of Tangier in the north of Morocco (35.76.252° N 005. 58.932° W).
5 fungi strains have been isolated from the root of ericaceous, Three of them are identified as ericoid mycorhizal fungi, and one was shown to belong to the *Phialocephala fortinii* and one to the genus *Cryptosporiopsis*, concerning actinomycetes, we have isolated 7 strains from ericaceous soil,
All those micro-organismes were tested for their ability to grow on a synthetic minimum medium (SMM) and milieu Nautyal (1966), demonstrated that all strains were able to grown in a synthetic minimal medium (SMM) containing TCP (tricalcic-phosphate) insoluble phosphate as sole P source.
The five fungi strain and two actinomycetes showed the most active growth and solubilization capability. These isolates were shown to be able to solubilize P in liquid cultures. The study of mechanisms involved in these weathering processes indicated that all fungi and actinomycetes produce siderophores and organic acids.
Keywords: endomycorhizia, Actinobacteria, ericacous shrubs, solubilization capability.

Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus

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Microbial mineralization and immobilization of nutrients strongly influence soil fertility. We studied microbial biomass stoichiometry, microbial community composition, and microbial use of carbon (C) and phosphorus (P) derived from glucose-6-phosphate in the A and B horizons of two temperate Cambisols with contrasting P availability. In a first incubation experiment, C, nitrogen (N) and P were added to the soils in a full factorial design. Microbial biomass C, N and P concentrations were analyzed by the
fumigation-extraction method and microbial community composition was analyzed by a community fingerprinting method (automated ribosomal intergenic spacer analysis, ARISA). In a second experiment, we compared microbial use of C and P from glucose-6-phosphate by adding ^{14}C or ^{33}P labeled glucose-6- phosphate to soil. In the first incubation experiment, the microbial biomass increased up to 30-fold due to addition of C, indicating that microbial growth was mainly C limited. Microbial biomass C:N:P stoi- chiometry changed more strongly due to element addition in the P-poor soils, than in the P-rich soils. The microbial community composition analysis showed that element additions led to stronger changes in the microbial community in the P-poor than in the P-rich soils. Therefore, the changed microbial biomass stoichiometry in the P-poor soils was likely caused by a shift in the microbial community composition. The total recovery of ^{14}C derived from glucose-6-phosphate in the soil microbial biomass and in the respired CO_2 ranged between 28.2 and 37.1% 66 h after addition of the tracer, while the re- covery of ^{33}P in the soil microbial biomass was 1.4e6.1%. This indicates that even in the P-poor soils microorganisms mineralized organic P and took up more C than P from the organic compound. Thus, microbial mineralization of organic P was driven by microbial need for C rather than for P. In conclusion, our experiments showed that (i) the microbial biomass stoichiometry in the P-poor soils was more susceptible to additions of C, N and P than in the P-rich soils and that (ii) even in the P-poor soils, mi- croorganisms were C-limited and the mineralization of organic P was mainly driven by microbial C demand.

The Expression Analysis of Plasma Membrane Aquaporin Gene EjPIP2 in *Eriobotrya japonica* After AM Fungi Inoculation

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The aim of this study was to clone a plasma membrane aquaporin gene EjPIP2 from *Eriobotrya japonica* cultivars 'Zaozhong 6', and to analyze its sequence characteristics, then to study the effects of AM fungi on the PIP expression pattern and water use efficiency(WUE). A pot experiment with seedlings of loquat cultivar ('Zaozhong 6'), two treatments (AM and NM) and five replicates were conducted. The leaf water use efficiency was measured by using LI-6400 photosynthesis determination system. The full-length cDNA of EjPIP1 was obtained by the technology of reverse transcription PCR (RT-PCR) and RACE. The cDNA sequence and the deduced amino acid sequence were analyzed by bioinformatics method. The expression patterns of EjPIP2 in leaves and roots by AM fungi inoculation were determined by real-time RT-PCR. Results showed that in the effective photosynthetic time(7:00—17:00), the water use efficiency of loquat leaves were significantly increased by AM fungi inoculation. Loquat plasma membrane aquaporin gene EjPIP2 (GenBank Accessin No. JX041626) was cloned. The full-length cDNA of EjPIP2 gene consists of 1 149 bp and contains a 846 bp open reading frame (ORF) encoding 286 amino acid proteins. Two highly conserved NPA (Asn-Pro-Ala, asparagine-proline-alanine) motifs of aquaporin were identified in EjPIP2. The homologue analysis revealed that the amino acids sequence of loquat PIP2 was highly homologous with other species, especially walnuts (*Juglans regia*), and grape (*Vitis vinifera*) which was up to 91%, and 88%, respectively. Real-time PCR analysis revealed that EjPIP2 could be expressed in different loquat tissues including root and leaf, and the relative expression in leaf was higher than that in root. Furthermore, under well-watered condition, the expression of EjPIP2 in loquat leaf was significantly down-regulated by AM fungi inoculation, while the expression of EjPIP2 in root was up-regulated. The water use efficiency of loquat leaves was significantly increased by AM fungi inoculation. A plasma membrane intrinsic proteins (PIPs) gene, designated as EjPIP2, was cloned from leaf of loquat (*E. japonica* 'Zaozhong 6'). And the expression of this corresponding gene in loquat leaves and roots were influen ced by AM fungi, which helped to improve water use efficiency of loquat seedlings after being inoculated with AM fungi.

Do soil type, rice cultivar and water management affect the bacterial denitrifying community of a paddy soil?

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Nitrate (NO_{3-}) can serve as electron acceptor in an anaerobic respiration called denitrification. The complete denitrification pathway converts NO_{3-} to N_2, therefore losing nitrogen from soil to the atmosphere. Many denitrifying microorganisms lack the nitrous oxide reductase enzyme, and produce N_2O as the final product of denitrification. Moreover, N_2O can also be produced by dentitrifiers with the complete set of genes, depending on several environmental parameters, particularly O_2 availability. As N_2O is a potent greenhouse effect gas, efforts are made to diminish its emissions. Rice paddies provide a suitable environment for denitrification to take place. Therefore, knowledge about how different agricultural practices on rice affect denitrifying populations is desirable. In this work, we conducted two greenhouse experiments where we analyzed the influence of soil type, rice cultivar and water management on the bacterial denitrifying community. We used the genes of the two known nitrite reductases (*nirS* and *nirK*) as PCR targets, and determined the abundance and population structure of these microorganisms by q-pcr and T-RFLP, respectively. In general, we found *nirK* numbers to be about 3 orders of magnitude higher than those of *nirS*. Abundance of *nirK* gene was not affected by rice cultivar nor water management. Only sampling date had a significant effect on *nirK*, samples taken at the beginning of the experiment (tillering stage) had lower *nirK* numbers than those taken at harvest (Tukeys, $p<0.05$). On the second experiment, *nirK* abundance was affected by soil type, higher numbers were found on the higher organic matter soil. On the first experiment, *nirS* was affected by sampling date with the same tendency found for nirK. Also, rice cultivar had a significant effect on nirS abundance, particularly on the earliest sample (tillering stage). On the second experiment, all three variables had a significant effect on *nirS* abundance. As well as for *nirk*, *nirS* was more abundant in the higher organic matter soil. Community structure of *nirS* denitrifiers was affected by soil type according to the multiresponse permutation procedure test but was not affected by rice cultivar. However, in the first experiment, population structure of *nirS* gene was affected by the sampling date. Indicator species analysis of *nirS* T-RFs found 3 T-RFs strongly associated to each soil type and one T-RF associated to cultivar “El Paso”.

Interactive effects of *Bacillus subtilis* and seaweed (kelpak)on the growth, metabolites and yield of potato (*Solanum tuberusom* L.) under glasshouse conditions

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Potato (*Solanum tuberosum* L.) is a worldwide cultivated tuber bearing plant which is the fourth main food crop in the world. However, the yield and profitability of this crop are currently low due to several factors including diseases and agronomic practices. Plant-growth-promoting bacteria and seaweed extracts are known to stimulate plant development and nutrition. In this study, the interactive effect of a local seaweed extract (Kelpak) and bacteria (*Bacillus subtilis*) was tested on three potato cultivars (BP1, Valor and Up-to-date) grown under glasshouse conditions. Certified potato seed tubers were surface sterilized and planted in autoclaved potting soil in plastic bags with four treatments namely Control (without bacteria and seaweed extract); Kelpak (amended with seaweed extract); *Bacillus* (inoculated with *B. subtilis*) and Kelpak & Bacillus combined. For each treatment, the germination rate and growth parameters (plant height and basal stem diameter), stomatal conductance and chlorophyll content were recorded at five-day intervals. Tuber yield (tuber weight and number per plant) for each treatment was determined at 90 days after planting. Mature leaves from each treatment were analyzed for secondary metabolites using Nuclear Magnetic Resonance (NMR) spectroscopy. Differences in germination rate were noted in response to the treatments with all cultivars having the highest rates in soils inoculated with *B. subtilis*. The treatment also significantly ($p<0.05$) affected plant height and basal stem diameter with plant inoculated with *B. subtilis* relatively taller compared to plant supplied with Kelpak only, which had the lowest height and stem diameter. In contrast, the control plant had more tubers and tuber weight per plant compared to the other treatments. The stomatal conductance was highest on the abaxial surface of the leaf in all cultivars and with significant differences in the chlorophyll contents in some treatments when compared to the adaxial surface. Analysis of NMR spectra using principal component analysis revealed differences in secondary metabolites produced in response to treatments in some cultivars but majority of the metabolites were common to all.

Effected of regular or concentrate vinasse on greenhouse gases emissions from soil with sugarcane

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Nitrogen (N) fertilization has been the main source of greenhouse gases (GHG) emissions to the atmosphere in sugarcane cultivation, mainly due to nitrous oxide (N_2O). In addition, some management practices of sugarcane production in Brazil may affect GHG emissions, such as the use of vinasse, a by-product of ethanol production, combined with N fertilizers. Alternatives to reduce these emissions are necessary because the main destination of vinasse is the soil, where it recycles nutrients and improve soil fertility. The objective of this study was to evaluate the N_2O losses during the sugarcane ratoon cycle after regular (V) and concentrated vinasse (CV) application before or along with mineral fertilization, and to determine the best time for application of vinasse aiming to reduce the GHG emissions. Besides determine the microbial functional groups of the N cycle and correlate with GHG emissions in soils. The experiment consisted of two application times of vinasse, T1: 30 days before N fertilization and T2: together with N fertilization, and two types of vinasse V or CV, amounting to 10 treatments and four replications. Nitrogen was added at a rate of 100 kg ha^{-1} (ammonium nitrate) and vinasse at 100 m3 ha^{-1} for V and 17 m3 ha^{-1} for CV. Nitrous oxide emission was evaluated for 230 days, with daily sampling the week after treatments application, and then every two days. Gas samples were analyzed by gas chromatography. Total soil DNAs will be used as templates for Real-time PCR for specific genes amoA (archaea, bacteria), *nifH*, *nirK* and *nosZ*. Data of cumulative emissions was submitted to ANOVA and means were compared by orthogonal contrasts ($P<0.05$). The highest N_2O emission was in the treatment of Vinasse with inorganic fertilizer applied together; the emission factor was 0.9% for N fertilizer and 0.7%. for CV. However, N_2O emissions from the fertilized plots without vinasse or when vinasse application was anticipated were 0.2 % and 0.3% of the N applied, values below the IPCC default value. The application of N together with V and CV increased GHGs emissions, possibly due to the presence of both readily available C and N. Therefore, a 1-month interval between application of vinasse and N seems to be a good strategy to reduce the N_2O emission. We will present results of the specific effects of sugarcane residues (V and CV) on abundance of the nitrogen cycle genes and their correlation with nitrous oxide emissions.

Nutrient limitation of soil microorganisms - effects of grassland land-use intensity

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The activity of microorganisms in soils is typically limited by the availability of carbon and microbial respiration increases when C resources as for example glucose are added. However for the growth of microbial populations the availability of additional nutrients, like nitrogen and phosphorous is required. Whether N and P are accessible for soil microorganisms and to which degree microbial growth is limited likely depends on soil type as well as on its nutrient status. Land-use influences the soil nutrient status due to the application of N and P containing mineral or organic fertilizers. We hypothesize that with increasing land-use intensity, i.e. increasing application of fertilizers soil microorganisms are less limited by N and P and that microbial growth is possible when easily available is present in soils. We investigated the influence of land-use intensity on soil respiration and the growth response of soil microorganims to C, N and P additions in soil samples from 50 grasslands with differentially intense long-term management. The grasslands are located in the Schwabian Alb region (southwest Germany) and form part of the German Biodiversity Exploratories (http://www.biodiversity-exploratories.de). First results showed that soil respiration, as well as microbial biomass increases with increasing land-use intensity. However, specific microbial respiration (respiration per unit microbial biomass) was negatively correlated with land-use intensity, indicating that a part of the higher microbial population in intensely managed grasslands is in an inactive status. Following our hypotheses, first results also indicate that nutrient limitation of microbial growth was more pronounced in less intensely managed grassland soils. We conclude that land-use intensity influence microbial growth response and their activity, which likely has consequences on organic matter turnover, C-storage potential and plant available nutrients in grassland soils.

Restoring the functional integrity of a Technosol with native organic materials

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Mine soils are hardly directly cultivated unless they are restored, but tantalite mine soils of western Rwanda are concomitantly cultivated alongside arable and marginal lands due to high population pressure on land. The farmers rely solely on farm yard manure (FYM) for plant nutrient supply. However, the contribution of FYM and native organic materials to biological mine soil quality has been least explored. The purpose of this study was to investigate the effects of FYM, biomass of *Canavailia braziliensis* and *Tithonia diversifolia*, and *Markhamia lutea* leaf litter on C and N mineralisation and microbial properties in a Technosol (unrestored mine soil) from western Rwanda. The laboratory experiment consisted of a Technosol and an arable soil with five treatments including (a) non-amended control soils, (b) FYM, (c) *Canavailia*, (d) *Tithonia* biomass, and (e) *Markhamia*. Fresh soils from each soil type were sampled at 20 cm depth, adjusted to 50% water holding capacity, amended and incubated for 12 weeks. Evolved CO_2 was measured weekly by alkali trap while mineralised N and microbial properties were analysed at 4, 8 and 12 weeks of incubation. The results revealed substantial ($p<0.01$) increases in CO_2 efflux, mineralised N, and microbial properties from amended soils compared to their non-amended counterparts. This was slightly higher in the Technosol than in arable soil. Canavalia and FYM mineralised N as opposed to N immobilisation in *Markhamia* and *Tithonia* treatments. Canavalia significantly ($p<0.001$) increased cumulative CO_2-C and mineralised N in non-amended soils by 320-360% and 54-126% respectively, microbial biomass C by 240-593% and N by 244-376%. Canavalia also showed the least percent decrease in microbial biomass C during the incubation period. *Markhamia* and *tithonia* treatments immobilised 36 to 61% of non-amended soils N whereas tithonia increased ($p<0.01$) ergosterol content by over 2-fold the amount in non-amended soils. In conclusion, the Technosol has high potential for quick restoration mostly because of pH (5.6), and cations from unweathered soil minerals compared to arable soil (pH 4.9). The performance of the amendments present opportunities for optimum use in mine soil restoration particularly through integrated use for a balance between C sequestration and slow nutrient release.
Key words: *Canavalia braziliensis*, *Markhamia lutea*, microbial biomass, N mineralisation, *Tithonia diversifolia*, tantalite mine soils

Identifying potential key players of N_2 fixation in European biological soil crusts

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Biological soil crusts are millimeter- to centimeter-thick assemblages of soil or mineral particles with cyanobacteria and other bacteria, archaea, microalgae, fungi, and possibly also lichens and bryophytes. These crusts form in any habitat where vascular plant growth is limited; they play an essential role in soil stabilization and reduction of soil erosion by wind or water, as well as atmospheric CO_2 and N_2 fixation. On a global scale, it has been recently estimated that biological soil crusts contribute ~30% of the total biological N_2 fixation in terrestrial ecosystems. Although the bacterial and archaeal communities in soil crusts have been studied by molecular methods for several years now, less is known about the diversity of diazotrophic (N_2-fixing) communities. Furthermore, most of the investigations on diazotrophs in soil crusts have been focused on arid or semi-arid locations, while our knowledge on biological soil crusts of temperate regions is still scarce.
We conducted $^{15}N_2$ incubation experiments on soil crust samples from four different European locations, which have been provided by the Soil Crust International project (SCIN). These sites represent typical European climate regions along a latitudinal and altitudinal gradient with varying mean annual temperature and mean annual precipitation, ranging from -3.0 °C and 2000 mm (Hochtor, Austria) to 18.5 °C and 240 mm (Almeria, Spain). We incubated soil crust samples for 24h in an artificial $^{15}N_2$ atmosphere and could detect N_2 fixation activity in all investigated sites by isotope ratio mass spectrometry (IRMS). These data revealed a heterogeneous pattern of N_2 fixation at the spatial scale within each site, with values ranging from near natural abundance to ^{15}N signatures of 254‰ vs. atm. air.
The diazotroph community composition of selected incubated crust samples was investigated using barcoded Illumina MiSeq sequencing of the marker gene for N_2 fixation – the dinitrogenase reductase (nifH). The nifH amplicon libraries of all crust samples were dominated by sequences associated with Cyanobacteria and Alphaproteobacteria; however, we detected differences of diazotroph diversity in different crusts on the genus level. On-going efforts focus on amplicon sequencing of nifH transcripts in these crust samples to elucidate the diazotroph community actively expressing the dinitrogenase reductase gene, which is potentially involved in N_2 fixation.

Recently identified microbial guild mediates soil N_2O sink capacity

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Abstract Nitrous oxide (N_2O) is the predominant ozone-depleting substance and contributes approximately 6% to overall global warming. Terrestrial ecosystems account for nearly 70% of total global N_2O atmospheric loading, of which at least 45% can be attributed to microbial cycling of nitrogen in agriculture. The reduction of N_2O to nitrogen gas by microorganisms is critical for mitigating its emissions from terrestrial ecosystems, yet the determinants of a soil's capacity to act as a source or sink for N_2O remain uncertain. To address whether the composition and structure of the N_2O reducing communities matters for N_2O consumption in soil, we analyzed the diversity of this guild amongst the different European soils by pyrosequencing. We demonstrated using structural equation modeling that the soil N_2O sink capacity is mostly explained by the abundance and phylogenetic diversity of a newly described N_2O reducing microbial group, which mediate the influence of edaphic factors. Analyses of interactions and niche preference similarities suggest niche differentiation or even competitive interactions between organisms with the two types of N_2O reductase. Using co-occurrence analysis, we further identified several recurring communities comprised of co-occurring N_2O reducing bacterial genotypes that were significant indicators of the soil N_2O sink capacity across different European soils.

Lasting influence of biochemically contrasting organic inputs on abundance and community structure of total and proteolytic bacteria in tropical soils

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The SOM field experiments in Kenya, which have been initiated in 2002 on two contrasting soils (clayey Humic Nitisol (sand: 17%; silt: 18%; clay: 65%) at Embu, sandy Ferric Alisol (sand: 66%; silt: 11%; clay: 22%) at Machanga), were used for exploring the effect of nine year annual application of biochemically contrasting organic inputs (i.e., *Zea mays* (ZM; C/N ratio: 59; (lignin+polyphenols)-to-N ratio: 9.8); *Tithonia diversifolia* (TD; 13; 3.5); *Calliandra calothyrsus* (CC; 13; 6.7)) on the soil bacterial decomposer community. Soil samples were taken at the onset of the rainy season before application of fresh organic inputs in March 2011. We studied the abundance (quantitative PCR) and community structure (T-RFLP analysis) of the total (i.e., 16S rRNA gene) and specifically proteolytic (i.e., npr gene encoding neutral metalloproteases) bacteria. Alterations of the soil microbial decomposer community were related to differences of quantity (i.e., soil carbon (TC)) and particularly composition of SOC, where mid-infrared spectroscopic (DRIFTS) information, and contents of extractable soil polyphenol (PP) and the newly introduced PP-to-TC ratio served as SOC quality indicators. For total bacteria, effect of organic input quality was minor in comparison to the predominant influence of soil texture. Elevated soil PP content, driven by polypheneol rich organic inputs, was not suppressive for overall bacterial proliferation, unless additional decomposable C substrates were available as indicated by PP-to-TC ratios. In contrast to the total bacterial community, biochemical quality of organic inputs exposed a stronger effect on functionally specialized bacterial decomposers, i.e., proteolytic bacteria. The npr gene abundance was depressed in the TD treated soils as opposed to soils receiving CC, and showed a positive correlation with soil PP. It was suggested that the high presence of lignin and polyphenol relative to the N content, in organic inputs was increasing the npr gene abundance to counteract most likely the existence of polyphenol-protein complexes aggravating protein degradation. We concluded from our study that integration of spectroscopic, geochemical (i.e., soil PP) and molecular soil data provides a novel pathway to enhance our understanding of the lasting effect of organic input quality induced SOC quality changes on bacterial decomposers and particularly proteolytic bacteria driving soil organic N cycling.

Microbial activity in the context of acid deposition – field manipulations with sulphur and/or nitrogen inputs to the forest soils

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Nitrogen retention capacity (N saturation) of terrestrial ecosystems was shown to be tightly related to their carbon balance. Cycles of both elements have been influenced to a large extent by anthropogenic deposition. The question, however, is how the cycles respond to different proportions of S and N compounds in acid input. The answer could contribute to our understanding of the unsystematic decrease of nitrate leaching observed after the decline of sulphur deposition. We aimed to evaluate individual and combined effects of S and N deposition on a range of key biochemical and microbial parameters of C and N transformations in forest soils (e.g. C & N enzyme activities, C & N microbial biomass, C & N mineralization, and DOM biodegradability). In 2013, we established two experimental plots in spruce and beech forest stands of the same elevation (784m a.s.l.), bedrock (paragneiss), and deposition history in the Ore Mountains (Czech Republic) to manipulate S and N inputs. In spring 2014 we started to apply S and N treatments and combination of both in randomised block design (eight doses per year, NH_4NO_3 50kg N $ha^{-1}y^{-1}$; SO_4^{2-} 50kg S $ha^{-1}y^{-1}$, control included). Immediately after the beginning of manipulations, soil acidity increased markedly in S and SN treatments of both stands (pH decrease of 0.5 units on average). Base cations together with H^+, SO_4^{2-} and Alex mobilized to the soil leachate. The DOC concentration started to decrease in S and SN treatments, while in the N treatment, no changes relative to control were observed. Nitrates rose in N and SN treatments, but it is not clear if they originate directly from the N addition or result from a shift in microbial N transformation. This will be disentangled using ^{15}N partitioning among soil N pools this year. The complex of microbial activity data have shown only minor changes so far. Nitrogen addition stimulated activities of all measured enzymes in the beech stand while only the cellulolytic activity (beta glucosidase & cellobiosidase) was enhanced in all treatments of the spruce stand. Microbial respiration decreased in all treatments at both stands compared to control. Other results are unsystematic for now. However, it seems that the acidification potential of S addition compared to rather nourishing effect of N treatment will be more and more influential in both stands as the study progresses.

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Influence of different clay minerals on the microbiome of soils and its functionality in simplified artificial systems

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The multitude of microhabitats and biogeochemical interfaces (BGIs) makes soil one of the most heterogeneous and complex ecosystems that microorganisms can inhabit. The formation of BGIs depends on the soils mineral composition, which in turn strongly influences the development of microbial communities and their functional traits.
In this study we used two artificial soils that consisted of sand and silt-sized quartz and varied in clay minerals with different swelling potential (montmorillonite and illite) to study the development of a stable soil microbiome and to investigate its functional traits. Both artificial soils were inoculated with microorganisms from agricultural soil and sterile manure as nutrient source at the beginning and after 562 days. The mixtures were incubated under controlled laboratory conditions for 842 days. After this period, total community DNA from three replicates per composition was extracted and used directly for the construction of metagenomic libraries. Paired-end shotgun sequencing was performed using the MiSeq Illumina platform.
The majority of assigned reads were related to Bacteria (93.7%); 2.1% to Archaea and 4.2% to Eukaryota. Principal component analysis revealed a clear clustering of different soils. Diversity analysis based on Shannon's index implies more stable community in montmorillonite compared to illite. While dominant phyla, including Bacteroidetes, Firmicutes and Proteobacteria are comparable, significant differences were detected among less abundant taxa - Caldiserica, Chlorobi and Gemmatimonadetes, indicating the importance of the rare microbial biosphere for the unique functional response pattern of soils differing in the soil structure and texture.
As the aggregate formation is of high importance during initial soil formation, we studied in detail the role of the soil microbiome for the production of EPS, which acts as glue and is thought to promote the aggregation. We used Hidden Markov models to identify key players that drive the production of EPS during soil development. We could detect significantly higher abundance of glycosyltransferase WcaA and WcaC, enzymes involved in colonic acid biosynthesis and components of PEP-CTERM/EpsH, exopolysaccharide-associated protein sorting system in illite. Immaturity of the illite ecosystem and ongoing soil formation may explain the higher abundance of EPS pathways when compared to montmorillonite.

Predicting temporal and spatial variations in bacterial phylogenetic and phenotypic community structure in glacier forefield chronosequences

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The forces driving spatiotemporal changes in microbial communities remain unclear. Most information and theory on community assembly arise from animal and plant ecology and extrapolation of these theories to microbes is challenged by difficulties in defining species and linking phylogeny and function and by assessing their enormous taxonomical and functional diversity.
In this study we have adopted an innovative and original approach to predict spatiotemporal changes in bacterial communities in three Alpine glacier forefields. These forefields represent a primary succession spanning 150 years, with distance from the receding glacier forefront as a proxy for time (soil age since deglaciation). Geographic distance between the three sites was 125, 200 and 325 km, accounting for the spatial component of our predictions.
We first hypothesised that soil and vegetation ageing drive bacterial succession. We predict that environmental parameters follow the same temporal pattern between glaciers, so that the succession of bacterial traits will be similar. We specifically predicted the changes in (i) bacterial taxonomy and (ii) specific functional genes, relative to abiotic stress tolerance or competition and biotic interactions, identified using KEGG database. We also hypothesised that, because of dispersal limits and potential allopatric speciation, phylogenetic divergence between two communities depends on the geographic distance between them. As a consequence, we finally predicted that the STAR (Species-Time-Area Relationship) applies to microbial communities, i.e. that diversity increased with both space and time, with synergistic interaction of both.
To test these predictions, we performed Illumina sequencing of the V3-V4 region of bacterial 16S rRNA genes and recorded a range of soil, plant and environmental characteristics predicted to affect bacterial assembly. Taxonomic community structure was found to depend on both soil age and geography, with communities in the oldest soils of the three glaciers consistently clustering, while communities in the youngest soils also depended on geographic localisation. Taxonomic affiliation is also being used to derive predictive metagenomes, using the recently developed PICRUSt software, with subsequent screening of these metagenomes observe changes in relative abundances of the functional genes of interest.

The functional profiles of soil microbial communities are determined by soil chemical properties but not community composition

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Soil microbes are the most important driver for the biogeobiochemical processes, and a particular microbial group is usually associated with some unique functions. The functional profiles of microbial community may be determined by community composition. On the other hand, soil chemical properties are the main environmental cues shaping the soil microbial community. However, the effects of soil chemical properties and microbial community on the functional profiles are far from understanding. In this study, we constructed special rhizoboxes with root chamber (RC) and rhizosphere space, in order that the rhizosphere soils with different distances from RC could be easily sampled. Soils in RC and in the near (Rn, 0-5 mm from RC) or the far rhizosphere (Rf, 10-15 mm from RC) were sampled. Soil chemical properties were measured. Microbial community composition was monitored with PLFA as the biomarkers. The functional profiles of microbial community were evaluated using the soil enzyme activities (SEA) and the quantification of functional genes.
Results showed that the contents of TOC and DOC were similar in RC, Rn and Rf. Available N content was lower in RC than in Rn or Rf for the gaminoid species but kept unchanged for the legume species. Available P content was similar in RC, Rn and Rf, while available K content was much different in the following order: Rf > Rn > RC. PLFA analysis showed that the microbial community was more diverse in RC than in Rn or Rf. Moreover, PCA indicated that the microbial community composition in RC was much different from that in Rn and Rf, with the latter similar to each other. SEA assay revealed that the activities of cellulose, cellobiosidase, b-glucosidase, nitrate reductase, leucine-aminopeptidase were not affected, however, the activities of b-glucosidase, b-xylosidase, decreased in Rn and Rf compared to RC. qRT-PCR of functional genes showed that the abundances of *AOB-amoA*, *nirS*, *nifH* and *alp* were affected. To demonstrate the relationship between functional profiles of microbial community and soil chemical properties or microbial community composition, Spearman's rank correlation (by Mantel test) analysis was conducted with R=0.2701 (P=0.001) for soil chemical properties and 0.1312 (P=0.078) for microbial community composition. This suggests that the functional profiles of soil microbial communities are determined more possibly by soil chemical properties than by microbial community composition.

Effect of biochar application to soil on soil microbial communities structure and feeding habits: a field study in Mediterranean soils

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Biochar is a variety of black carbon produced by thermal decomposition of biomass in total or partial absence of oxygen. alBiochar is alleged to be chemically inert when applied to soil mainly due to the abundance of condensed aromatic structures. Based on this recalcitrance, soil conditioning with biochar has been proposed as a strategy for carbon capture and storage. But laboratory incubations and pot experiments demonstrate that a certain proportion of the biochar-C may be available to soil microbial communities that, at the same time, could be substantially modified in size, structure and functions. Biochar microbial decomposition rate depends on original feedstock and production method and everything suggests that local soil and climate characteristics would be crucial for its preservation in soil over time. However, there is an almost total lack of information about biochar decomposition in field conditions, due to its relatively low decomposition rate and the the resulting need for long term monitoring.

We are working to state the ability of soil biota to decompose biochar in Mediterranan soils and to assess effects of biochar on soil microbial communities. To do so, we cultivated corn (*Zea mays*), a C4 plant, and made biochar of it by slow pirolysis. In July 2013, we applied this char to a vineyard whose soils have been historically cultivated with C3 plants, at a dose of 5 MgC ha^{-1}. C3 and C4 plants show different $^{13}C/^{12}C$ isotopic ratios (^{13}C: -22 to -30 ‰ for C3 and -10 to -19 ‰ for C4) and the isotopic ratio of soil microbes will reflect the isotope rate of their diet.

We have been sampling three amended plots together with three more unamended control plots for two years, four times a year with seasonal periodicity. Samples are being studied for soil microbial community size and structure by PLFA analyses and the main diet (C4 biochar or C3 soil organic matter) of each microbial group is being assessed by isotopic analysis of the main microbial PLFA markers. We show here the results of the first year of monitoring. Biochar application to soil caused a significant decrease in the biomass of microbial PLFAs, and the proportion of fungal and gram-positive bacteria PLFA markers was significantly different in control and treatment plots. We also show our first data on feeding preferences of soil microbes in both biochar amended and unamended plots.

Metal tolerant, plant growth promoting soil bacteria protected plants against the toxic effects of heavy metals (Cd, Cr, and Ni)

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Bacterial inoculation may influence Brassica juncea growth and heavy metal (Ni, Cr and Cd) accumulation. Three metal tolerant bacterial isolates (BCr3, BCd33 and BNi11) recovered from mine tailings, identified as *Pseudomonas aeruginosa* KP717554, *Alcaligenes feacalis* KP717561 and *Bacillus subtilis* KP717559 were used. The isolates exhibited multiple plant growth beneficial characteristics including the production of indole-3-acetic acid, hydrogen cyanide, ammonia, insoluble phosphate solubilization together with the potential to protect plants against fungal pathogens. Bacterial inoculation improved germination of seeds of B. juncea plants in the presence of Cr, Cd and Ni, as compared to the control treatment. Compared with control treatment, soil inoculation with bacterial isolates significantly increased the amount of soluble heavy metals in soil by 51% (Cr), 50% (Cd) and 44% (Ni) respectively. Pot experiment of *B. juncea* grown in soil spiked with heavy metals ($NiCl_2$, $CdCl_2$, and $K_2Cr_2O_7$) revealed that inoculation with metal tolerant bacteria not only protected plants against the toxic effects of heavy metals, but also increased growth and metal accumulation of plants significantly. These findings suggest that such metal tolerant, plant growth promoting bacteria are valuable tools which could be used to develop bio-inoculants for enhancing the efficiency of phytoextraction.

Effects of cropping system, depth, and sampling time on soil microbial communities

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We investigated how conventional (corn-soybean rotation) and diversified (corn-soybean-oats/alfalfa-alfalfa) cropping systems, soil depth (0-10 or 10-20 cm), and time of sampling (pre-planting, corn growth phase, mature corn, post-harvest) influence soil microbial communities. After collecting samples in the field, total DNA was isolated for amplicon sequencing of the V3-4 region of the 16S rRNA gene using the Illumina MiSeq platform. Our data reveal that microbial community structure is strongly affected by the time of sampling, and to some extent by soil depth and cropping system. Additionally, corn appears to be the main driver in microbial community shifts suggesting an active selection of its associated microbiome. Our findings should enable a better understanding of plant-microbe interactions contributing to maize health and environmental influences on those communities, including the biogeochemical processes they mediate.

Effects of temporal pH shifts on ammonia oxidiser community structure and function

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Soil nitrification, the oxidation of ammonia to nitrate, is and driven by bacterial and archaeal autotrophic ammonia oxidisers (AOB and AOA) that carry out the first, rate limiting, step of oxidising ammonia to nitrite. Previous work has suggested that adaptation and selection in AOA and AOB communities is, to some extent, pH driven. Acidophilic, acido-neutral, and alkalinophilic groups have been identified by environmental surveys of *amoA* genes. These studies of the role of pH in determining ammonia oxidiser community structure and activity have largely relied on spatial pH gradients. In many managed soil systems (e.g., agricultural systems) edaphic factors (e.g., pH, N concentrations) vary widely temporally and the implications of short term temporal shifts in factors thought to govern oxidiser community structure, and therefore our ability to manipulate edaphic factors to direct community structure, are not well understood. We investigated the roles of pH in driving nitrifier activity (potential) and community structure over a crop growing season (6 sampling points) in agricultural soils by comparing unamended soils with soils amended with lime to create a temporal pH gradient. Liming induced a rapid and sustained change in the pH of surface soils (0-10cm), with pH in these soils increasing from 4.8 to 6.5, while in subsurface soils pH increased to a lesser degree after liming (4.3 – 4.5). After liming, potential nitrification rates increased significantly throughout the production season in both surface and subsurface soils. TRFLP analysis of total bacterial and archaeal communities showed significant partitioning of the broader communities with soil depth, pH treatment and time, suggesting that microbial communities respond rapidly to changes and that temporal variation in community structure is an important, if often overlooked, factor in assessing microbial diversity patterns. These changes were greater for bacterial, than archaeal, communities. We then utilised *amoA* gene microarrays to investigate specific AOA and AOB community responses to temporally induced pH changes. Despite significant changes to ammonia oxidiser function, we saw only very weak changes in community structure of AOA and AOB, suggesting that over shorter temporal periods soil communities are resilient to environmental change and that niche partitioning of ammonia oxidiser communities is likely to be spatially, rather than temporally, governed.

Response of Bacterial and Archaeal nitrifying populations to changing landscapes

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Nitrogen is an essential primary plant nutrient and its application to farmland systems as a fertiliser has major impacts on crop yields and in maximising the economic returns. However non-targeted approaches are not only a loss of a vital resource but also may result in damaging N losses to the environment either as ground water run-off and possible eutrophication of water sources or as potential greenhouse gases. The regulation of N cycling within soil is mainly dependent on a diverse and abundant group of soil microorganisms.
Nitrification, the oxidation of ammonia to nitrate is performed by specific groups of ammonia oxidising bacteria and archaea (AOB, AOA). The initial step the conversion of ammonia to hydroxylamine is the rate limiting step in nitrification and is catalysed by distinct ammonia monooxygenase (*amoA*) enzymes in the two kingdoms. Both AOB and AOA are slow growing with AOB being obligate chemoautotrophs, however not all AOA are obligate chemoautotrophs. They also appear to respond differently to ammonia with AOA having lower ammonia saturation and inhibition constants compared to AOB. This may be reflected in differences in their relative abundance within agricultural and non-agricultural settings and their impact on nitrification during and post fertiliser applications.
Here we present the analysis of both the relative abundance and distinctive phylogenetic profiles of AOB and AOA populations from contrasting agricultural treatments (arable, bare-fallow and grassland, from the Highfield trial site at Rothamsted) derived from metagenomic and metatranscriptomic data sets. The analysis is allowing a greater understanding of the roles of these two nitrifying communities within an agricultural setting, to inform land management strategies and enable better control of the terrestrial nitrogen cycle.

Characterization of the bacterial processes responsible for zinc solubilization in wheat rhizosphere

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Zinc (Zn) deficiency in human populations is recognized as a global nutritional problem. To address this issue, biofortification strategies aim at increasing the bioavailable micronutrient density of the edible parts of plants. The success of these strategies is heavily dependent on the size of the phytoavailable Zn pool in soil. Chemical parameters controlling Zn phytoavailability are well identified (e.g. soil pH, calcium carbonate content). However, only little is known on the ability of soil bacteria to affect soil available Zn. Only a few strains belonging to the genera *Bacillus*, *Gluconacetobacter*, *Pseudomonas*, and *Acinetobacter* have been reported as Zinc Solubilizing Bacteria (ZSB) (i.e. bacteria that can release Zn from scarcely soluble Zn salts) and the bacterial processes controlling Zn solubilisation remain poorly investigated.
The objectives of this study are to (i) isolate zinc solubilizing bacteria and (ii) characterize the underlying solubilization processes under in vitro conditions. Soil samples from wheat rhizosphere were collected from a long term-field trial in Switzerland. Rhizosphere soil suspensions were spread on microbiological media supplemented with glucose and zinc oxide. In total, 156 colonies were identified as ZSB (i.e. halo forming colonies). All the isolates were characterized based on the comparison of their Matrix Assisted Laser Desorption/ionization Time Of Flight mass spectrometry spectra (MALDI-TOF MS). Among the 156 ZSB isolates, 85 were found to have distinct – strain specific – MALDI-TOF MS spectrum. The 85 strains were characterized for siderophore production on Chrome Azurol (CAS) agar. Finally, solubilization assays with two zinc substrates (i.e. zinc oxide or zinc carbonate) and different carbon sources commonly found in plant rhizosphere (glucose, malic acid and glutamate) were performed.
This work will provide new knowledge on zinc solubilizing bacteria and strengthen our understanding of the mechanisms controlling zinc phytoavailability at the soil-plant interface.

The influence of soil tillage on microbial communities changes along the soil profile

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Soil microorganisms are abundant and diverse, and can have both beneficial and adverse effects on crop growth. Some, such as plant-growth-promoting rhizobacteria and mycorrhizae, are well known to favor crop productivity and plant health. They are notably involved in key processes such as improving plant nutrient acquisition, stimulating plant growth and protecting plants against pathogens by producing bioactive substances. Conversely, agricultural practices such as reduce tillage are known to influence the physical and chemical properties of soil. As a consequence, the abundance and diversity of soil microorganisms may change, which in turn may impact crop growth. In addition, the agricultural practices could influence soil properties in relation with depth, and thus modifying microbial pattern along the soil profile.
This study aims to investigate the influence of tillage practice on microbial community composition at two ranges of depth: 0 to 5 cm and 15 to 20 cm. The first corresponds to the tilled layer under reduced tillage (RT) and the second corresponds to the plowed layer under conventional tillage (CT). High throughput sequencing was performed to sequence microbial DNA, bioinformatics tools were used to process the data and multivariate analysis was conducted to determine the difference in microbial communities at both ranges of depth according to the tillage practice.
Results demonstrate that microbial communities are differently shaped under RT and CT at both depths. Indicative taxa of each soil condition have been identified. They could have beneficial or deleterious effect on soil health and could explain difference in crop production under RT and CT.

Soil bacterial community under integrade production system at biomes savamma ams amazon

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The integrated production systems have been pointed as a sustainable alternative in use of land, therefore, study the microorganisms from soil is remarkable, since they play an important participation under the aspects of physic and chemical properties from soil The objective of this study was to evaluate the impact of integrated agricultural production systems through the bacterial soil communities in a transition area of Savanna and Amazon rainforest. The experimental field belongs to Embrapa Agrosilvopastoral (Sinop/MT) and three monoculture systems (Eucalyptus (E), Crop (C), Pasture (P)) and three integrated systems (ECI, PI, ECPI) arranged in four blocks were evaluated plus a Native forest as area of reference. Soil samples were collected at depth 0-10 cm in the wet and dry season in 2012 and using Illumina high-throughput sequencing of the 16S rRNA gene, the bacterial composition was determined. The result in the sequencing performed showed the greatest abundance were classified as phyla Firmicutes, Actinobacteria and Proteobacteria. The Firmicutes correlated with the Crop in the rainy period and in the dry only ECPI and Forest. For five classes corresponding to the three phyla, the Crop stood out with the greatest fluctuations in their relative abundance compared to other treatments. In cluster analysis by gender in the rain, only the Forest and the ECPI had no similarity with the other treatments but in the dry the two were grouped with the Forest and EPI. Therefore, the bacterial community in the soil integrated systems was sensitive to management systems with only two years of experimental deployment. The ECPI showed greatest similarity of bacterial structure with Native forest. Crop had shown fluctuations in the relative abundance in the two seasons showing an unsustainable production system under changes in microbial composition of the soil. Otherwise, as expecting the ECPI had the most similar behavior of the bacterial community from a sustainable environmental, giving credit that integrate system can change the future of world agriculture.

Rhizosphere microbiome, plant community and soil nutrient availability - a new approach to survey the bacterial assemblage in soil

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The soil nutrient content directly affects the composition and functioning of the soil microbiome. Besides this, the nutrient availability also strongly influences the performance of plants, which additionally affects the microbial community structure due to differences in rhizodeposition quantity and quality. In order to determine the relative impacts of nutrient availability and plants on the assemblage of the soil microbial community we set up an experiment using four soils from a centenary fertilization experiment. These soils, which highly differed in their nutrient content and also in their microbial community structure, were gamma sterilized and cross re-inoculated in a factorial design with a subsample of the four soils (1:6 wt/wt), in order to examine the responses of the four different microbial communities to changing nutrient conditions. In parallel, using the same soils and experimental design, we established a pot experiment with a mixed grassland community. We examined in each treatment the dynamics of the soil bacterial community structure as well as of the morphological and physiological plant traits in the pot experiment over a period of six months. Combining the results of both experiments allowed us to study the relative impacts of nutrient availability and plant growth on the soil microbial community. The nutrient availability strongly affected the soil community structure as well as the plant performance. In addition, notable shifts in the rhizosphere microbiome were also observed at specific plant growth stages and several bacterial OTUs were detected in the presence of plants which have not been found in the control condition without plants. These findings demonstrate the pivotal role of plants and their development stages in shaping the soil microbiome, which in turn, is one of the major driving factors in plant-soil feedback. Moreover, since sterilization creates a new soil environment for the inoculum, (i) it provides a relatively heterogeneous, physical model system to compare microbial community development and (ii) it offers the opportunity to monitor which bacterial species are involved in the plant-microbe interactions.

Digestate and fly ash applications in agricultural soils: impact in the biomass and biodiversity of fungal communities.

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The biogas production via anaerobic digestion of biomass and biomass combustion activities generate a large amount of residues usually known as digestate and fly ash, respectively. Both residues have shown to have a huge amount of organic matter and macro and micro-nutrients which make possible its use as potential soil improving amendments. The amendments applications can represent a good strategy for the maintenance of soil health which is essential for a sustainable agricultural production. Moreover, amendments addition can considerably improve the soil microbial biomass and diversity. Soil fungi represent one of the largest proportions of microbial biomass in soils and play an important role in the functionality and maintenance of soil since they are involved in the transformation of organic matter, C and N storage, and cycling of nutrients. For this reason, it´s essential to determine soil fungal responses when digestate and fly ash are applied into soils. To archive this objective, a Chernozem soil was incubated with digestate (100 Ma kg^{-1}) and fly ash (15 Mg ha^{-1}) and soil samples were collected after 30 and 60 days. The soil fungal biomass was measured as the number of 18S rRNA gene copies quantified by real time qPCR. The application of digestate provoked a slight enhancement in the copies number of 18S rRNA at 30 days, but it was detected a higher increase after 60 days of treatment. Meanwhile, no changes were detected in soil samples treated by fly ash at 30 and 60 days. The analysis of PLFA (18:2?6,9) in soil samples treated by digestate shown a significant increase in this parameter after 60 days of incubation. No changes were detected in presence of fly ash. The ergosterol content was also measured revealing an enhancement after 30 and 60 days of incubation with digestate, whereas fly ash did not modify this content. Fungal community diversity and abundance was analysed by PCR-DGGE fingerprinting. The fungal DGGE profile of all treatment was complex, with large number bands. The dominant bands were similar in all lanes except for variations in density. Different indices were calculated from analysis of the DGGE profiling. Digestate application provoked an enhancement in Shannon and Richness index (Hf and Jf) at 30 days, while Evenness index was increased after 60 days (Sf). Fly ash increased Hf, Sf and Jf at 30 days, but a decline was observed after 60 days.
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Does Land-Use Intensity Influence Microbial Resource Partitioning and Microbial Colonization Strategies of Organo-Mineral Complexes in Grassland Soils?

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The detritus- and rhizosphere, as well as minerals and organo-mineral complexes are microbial hot spots in soils. The colonization of these microhabitats depends mainly on biotic and abiotic soil properties. Nevertheless, mechanisms driving initial colonization processes of mineral surfaces and microbial feeding preferences are poorly understood. Further, there is still a debate whether bacteria, archaea and fungi start to feed on root litter resources at the same time or whether their activity occurs at different successional stages. Two questions were addressed within the Biodiversity Exploratories (http://www.biodiversity-exploratories.de): (1) Who are the key players in the detritivore pathway in grassland soils under different land-use intensity, and (2) Who is able to colonize the mineral surfaces and profits most from these resources? A randomized microcosm field experiment (4 x 4 m) was established on 10 different grassland sites at the Swabian Alb (Baden-Wuerttemberg, Germany) in September 2014. Experimental sites differ in land-use intensity (LUI) with 5 sites with low (grazed or mown once a year) and 5 with high (fertilized, mown and grazed) LUI-Index. Microcosms were filled with a site adapted mineral mixture consisting of: 71.4% illite, 9.6% goethite, 17% silt sized and 2% sand-sized quartz, and double labeled roots of Dactylis glomerata/ Lolium perenne (13.1 atom% ^{13}C and 31.1 atom% ^{15}N). Sampling of microcosms, adjacent soil and plants were performed after 1, 2, 6 and 12 months. First results showed an increase of microbial biomass over time which was slightly higher under high LUI (14 %) than under low land use intensity (8 %) verifying the early colonization of mineral surfaces. LUI did not change enzyme activities (C, N and P cycling) associated to mineral surfaces within the first two months, but changed ß-glucosidase and phosphatase activities in the bulk soil. Further analyses using isotopic techniques (^{13}C microbial biomass, ^{13}C PLFAs) will allow following the carbon flow from the added substrate into different groups of microorganisms associated to mineral surfaces. Molecular techniques (qPCR) will quantify possible changes of microbial community structure at high taxonomical level.

Plant genotype control over the recruitment of the tomato fungal microbiota

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Plant genotype was found to be less important than soil type in defining the composition of the bacterial plant microbiota [Lundberg et al. 2012 Nature 488 (7409): 86–90; Bulgarelli et al. 2012 Nature 488 (7409): 91-95]. However, given the unique biological and ecological features of fungi, recruitment of the fungal microbiota may deviate from the relationships documented for bacteria. For instance, due to the mycelial growth form, the distinction among the different rhizosphere compartments (root surface/tissues vs rhizosphere soil) may be less clear-cut for fungi than for bacteria.
Within the framework of a project combining meta-barcoding and systems biology to investigate the interactions between different tomato cultivars and their root mycobiota (Mycoplant), we addressed the relative impact of soil type, cultivar-specificity, and pathogen presence over the recruitment of the fungal microbiota. Tomato genotypes either susceptible (Moneymaker, Cuore di Bue) or resistant (Heinz, Battito) to *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were grown in two different soils under greenhouse conditions. The two resistant cultivars had also been inoculated with the pathogen (104 FOL conidia/ml). Approx. 900,000 ITS2 amplicons, assigned to 1128 OTUs, were analyzed. Both soil type and plant genotype significantly affected the structure of the root mycobiota (one- and two-way PERMANOVA, Jaccard distance measure). Generally, the effect of soil type on the structure of the fungal communities was higher than the effect of the plant genotype. However, in the presence of the pathogen, the magnitude of the impact of the two factors was comparable (variance partitioning, R vegan package). This finding indicates greater plant control over the fungal microbiota in a modified biological environment.

Community assembly processes of N_2O reducing prokaryotes in the rhizosphere-effect of edaphic factors and plant species

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Terrestrial ecosystems account for the major part of the emissions of the greenhouse gas nitrous oxide (N_2O) and emissions are increasing due to use of fertilizers in agriculture. The only known biotic sink is the N_2O-reductase, encoded by the *nosZ* gene, with Clade I mainly found among denitrifiers and Clade II common in non-denitrifying N_2O reducers. The rhizosphere is hotspot for denitrification, but whether the rhizosphere of different plants select for denitrifying communities that both produce N_2O and those capable of only reducing N_2O to N_2, is not known. To determine the effect of plants, plant species and soil type on the activity, abundance and community assembly processes of N_2O reducing communities in rhizosphere soil and on root surfaces, a pot experiment using a clay and a sandy soil planted with either sunflower (*Helianthus anuus*) or barley (*Hordeum vulgare*) was conducted. Denitrification and N_2O production rates in rhizosphere soil were not affected by plants, but N_2O production was detected on barley roots. Using quantitative PCR, we demonstrate that *nosZ* Clade I was favoured on the root surfaces, whereas it was the opposite for *nosZ* Clade II. This indicates different niches for Clades I and II and that plants, irrespective of species, selected for denitrifiers with a complete pathway. Phylogenetic analyses based on both NMDS of UniFrac distances and edgePCA show that the N_2O reducing communities of both *nosZ* clades differed according to soil and sample type. No plant effect could be observed in rhizosphere soil, but there was a significant plant-species effect on root-surface communities for *nosZ* Clade I. Net relatedness index inference showed that Clade I communities were less phylogenetically clustered on the root surface samples than in the soil, suggesting either competition or neutral community assembly processes on root surfaces. For *nosZ* Clade II, a higher degree of clustering was detected in both soil and on roots in the pots with sandy soil indicating a stronger effect of habitat filtering resulting in communities with more closely related members compared to the clay soil. The latter could be an effect of relatively more niches in the sandy soil. Overall our results indicate that *nosZ* Clade I and II have different niches and soil type overrides plant species considering diversity, structure, and functioning of N_2O reducing communities in the rhizosphere.

Pathogen-induced shifts in exudation alter the rhizosphere microbiome

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Plant roots are associated with dense microbial communities that form a shield against pathogens. Before causing disease, pathogens must break this line of defense. We hypothesized that this may occur via manipulation of plant exudates, a mix of labile carbon sources and bioactive molecules involved in selecting and feeding microbial communities. In this study, we showed that infection with the pathogen Ralstonia solanacearum resulted in an increased exudation of the phenolic compound caffeic acid. Caffeic acid inhibited R. solanacearum growth and promote PGPR Bacillus amyloliquefaciens T-5 growth in a dose-dependent manner. We applied caffeic acid and secretion during the interaction between the root of tomato and R. solanacearum to a soil. The structure of rhizosphere bacterial communities were then monitored by sequencing the 16S rRNA gene. Our analyses reveal that all the secretion application treatments significantly influenced the soil bacterial community structure and caffeic acid played an important role in modulating the soil bacterial community structure when tomato roots were challenged by R. solanacearum. These results imply that secretion of root–bacteria interaction regulate soil bacterial community structure and phenolic compounds act as specific substrates or signals for a large group of microbial species in the soil.

Soil Microbial Phosphorus Dynamics are Affected by Cover Crops and Minimum Tillage

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Phosphorus (P) constitutes an essential but non-renewable resource, that may restrict plant growth in many environments. The soil microbial community represents the key performer controlling the cycling of organic P, a major pool in the soil. However, little is known about its mineralization and availability to microbes. In order to reduce unnecessary fertilizer inputs and associated environmental pollution, alternative management strategies have to be developed. Cover crops and reduced tillage are both used in conservation agriculture, revealing themselves as a promising bundle of techniques with additional benefits for the agroecosystem. Our present project aims to study the influence of these techniques on the soil microbial community and the associated P dynamics, with a special focus on phytate, a recalcitrant organic P-compound. The samples proceed from a field experiment at the agricultural station in Tachenhausen (South-Western Germany), where the effect of minimum tillage and the presence of cover crop mixtures is assessed in a blocked design.
As P constitutes a limiting nutrient with low availability, the P content of the soil microbial community is hypothesized to be tightly coupled to the availability of inorganic and organic P pools.
We aim to elucidate the relationship between P limitation for microbial growth, assesed via respiration microcompensation system with substrate additions, the soil microbial biomass P content, determined by hexanol fumigation-extraction and the hydrolyzability of soil organic P using excess addition of enzymes to soil samples.
The results will support our understanding of the underlying mechanisms of the P cycle, enabling farmers to manage the limited P resources more efficiently by means of the application of advanced sustainable farming techniques.

Abundance and activity of soil microbial communities revealed by metagenomics and metatranscriptomics

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Soil microbial communities are notoriously difficult to study due to the presence of little-known groups that are difficult to culture and the complex nature of soil. Metagenomics, the analysis of DNA extracted directly from soil, avoids the need for laboratory culture and provides estimates of the relative abundance of difference groups. In metatranscriptomics, RNA is extracted to detect which genes from which groups are active in soil.

Long-established field experiments at Rothamsted Research have provided a valuable resource for investigating the effects of different land management and fertilizer regimes. These include comparisons between unperturbed grassland and regularly tilled soil under cereal cultivation or without plants, different nitrogen inputs, and variations in soil properties including pH. We have used 16S rRNA gene amplicon sequencing to reveal how bacteria and archaea respond to long-term treatments, augmented by full metagenomic sequencing of selected soils. This is complemented by functional measurements and metatranscriptomics analyses.

Results show certain groups are universally present at high abundance whilst others respond to soil treatments. Gene expression measured by metatranscriptomics is more variable and suggests that the most abundant groups are not always more active. Together, this data provides a picture of the microbial ecology in soils under typical arable agriculture and indicates the consequences of changing land use.

Soil microbial diversity patterns at Sites of the Swiss Soil Monitoring Network

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The Swiss Soil Monitoring Network NABO was established in the 1980ies to detect temporal changes of soil quality. About 100 soil monitoring sites were defined across Switzerland covering various land uses. Since microorganisms in soils are essential for many soil functions – such as nutrient cycling and storage as well as degradation, information on soil biological properties is crucial for soil monitoring networks. In this context, NABO has initiated monitoring of soil biological parameters for a sub-set of its long-term observation sites. Since 2012, every spring soil samples (0-20 cm) are collected at 30 NABO-sites (10 arable, 10 grassland and 10 forest sites). They are analysed for soil microbial biomass with substrate-induced respiration (BM-SIR) and chloroform fumigation-extraction (BM-FE), basal respiration (BR) and DNA-quantity. The soil microbiological diversity of the 30 sites is assessed by applying novel amplicon-based high–throughput DNA sequencing (HTS). Soil properties such as pH, C:N-ratio and bulk density are determined from the same soil samples. First results show that in general, arable soils contain less biomass than grassland and forest soils and that the different biomass measurements (BM-SIR, BM-FE and DNA-quantity) show a good positive correlation of 0.71 – 0.85 (Spearman). The comparison of the repeated measurements also shows that the BR-, BM-FE- and BM-SIR-contents in grassland and arable soils are in good agreement. In 2013, BR was 92%, BM-SIR 103% and BM-FE 98% of the contents of 2012. Forest soils differed more between the two years, e.g. at one site BM-FE-content in 2013 was 50% lower than in 2012. DNA quantities in grassland and arable soils of 2013 were lower than those of 2012 (61% lower in average). DNA quantities in forest soils were again more variable. In general, the results of the forest soils reveal a higher variability within the same year as well as between the years. All parameters analysed, except pH, show significant correlations (p-value < 0.01). The sites have been sampled from 2012 to 2015. This unique data set of repeated measurements within a longterm monitoring system allows us both, to determine the mean ranges of soil biological parameters and to reveal spatiotemporal patterns of the soil microbiota. The interaction among site properties and soil biological characteristics will be assessed with multivariate analyses of metadata from the NABO sites, such as management, nutrients, climate etc.

Field-scale spatial variation in co-occurrence patterns of ammonia and nitrite oxidizing communities.

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Nitrification is a principal driver of nitrogen loss from agricultural soils, resulting in nitrate leaching into ground water as well as increased emission of greenhouse gases in the form of nitrous oxide (N_2O). Previous work examining the spatial distribution of nitrifier community diversity and abundance in relation to edaphic factors have focused primarily on ammonia oxidizing bacteria (AOB) and archaea (AOA), which perform the first step in the nitrification pathway. In contrast, substantial knowledge gaps exist on the relevance of differences in nitrite oxidizing bacterial communities (NOB) performing the second step in the pathway, and whether non-random associations exist between NOB, AOB and AOA that contribute to spatial patterns of nitrifying community abundance and diversity. To address this, we explore the interaction between these communities in relation to spatial patterns of edaphic factors across a 44-hectare farm. The diversity and abundance of AOA and AOB communities was determined by PCR amplification of the ammonia mono-oxygenase gene (*amoA*) variants for both communities, while recently developed molecular probes (Pester et al., 2013) were used to assess NOB communities. Variance partitioning of generalized unifrac distances revealed that variation in the community structures of NOB and AOB were more influenced by biotic interactions, either isolated or in concert with spatial or edaphic factors, than by spatial or environmental variability alone. In contrast, the majority of variation in AOA community structure was explained by spatial and edaphic factors. The importance of biotic interactions was reflected in subsequent network analysis of co-occurring AO and NOB groups that identified distinct modules of nitrifying communities occupying disparate regions of the field site, and whose abundance varied with different soil factors. These results demonstrate the importance of accounting for biotic interactions in defining the niche space of functional communities at scales compatible to management strategies.

Exploring rhizobacterial community composition associated with plants grown in Chilean extreme environments using 16S rRNA-based molecular approaches

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Bacteria associated with natural vegetation from extreme environments have been scarcely studied. Chile is a long country with diverse undisturbed extreme environments where native plants are highly adapted to local conditions. Here, we explored the bacterial community structures in the rhizosphere of plants grown in Atacama Desert (ATA; driest and oldest desert on the earth), Andes Mountain (AND; Nature Reserve Huilo Huilo) and Antarctic (ANT; South Shetland Islands). Rhizosphere soils were collected from two plant species per location and bacterial communities were investigated by denaturing gradient gel electrophoresis (DGGE) and 454-pyrosequencing of 16S rRNA genes. Sequencing of representative bands from DGGE gels revealed the presence of Proteobacteria and Bacteroidetes phyla in all rhizosphere soils. By using 454-pyrosequencing, Proteobacteria (22~96%), followed by Actinobacteria (2~40%) and Bacteroidetes (0.01~38%), were dominant phyla in all rhizosphere soils. However, non-metric multidimensional scaling (nMDS) analyses based on both molecular approaches showed differences in bacterial community structures between studied extreme environments. Both approaches also showed differences between plant species. At class level, these differences were mainly attributed to Gammaproteobacteria (particularly Stenotrophomonas in ATA) and Alphaproteobacteria (particularly Burkholderia AND and ANT). However, how each rhizosphere bacterial group may contribute to survival and tolerance of native plants in each extreme environments remain unclear and further studies are still needed.
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Influence of soil management history on microbial N_2O production and reduction

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N_2O is a potent greenhouse gas, with an atmospheric half lifetime of 113 years, which also contributes to ozone layer destruction. Agriculturally managed soils hold for the gross of anthropogenic N_2O release. In order to develop effective mitigation strategies a detailed understanding of processes and mechanisms leading to N_2O formation and reduction in croplands is needed. As cycling of fixed N in soils is almost entirely controlled by microbial activity it is crucial to understand the long term impact of soil management on N cycling microbial communities.
Here we present results of an incubation study in which the impact of soil management history on N_2O emissions potential and denitrifying microbial communities was assessed. Therefore, soil samples from the DOK trial in Therwil/BL were taken, fertilized with $NH_4{}^{15}NO_3$ and incubated under controlled conditions at 80%WFPS. Long term organic (BIOORG) and conventional (CONMIN) management practices had been compared together with non-fertilized control (NOFERT).Apart from monitoring N_2O emissions qPCR analysis of functional genes was employed to quantify functional markers for denitrification and N_2O reduction on DNA and cDNA level. In order to complement molecular biological data ^{15}N tracing technique was used to determine N_2O/N_2 product ratio and fertilizer derived sources of N_2O emissions.
Results show increased N_2O emission potential of organic management history. Higher N_2O/N_2 product ratios indicate less complete denitrification in this treatment. Distinct activity patterns of denitrifying and N_2O reducing functional gene support this finding although only weak correlation to process rates has been observed. DNA analysis proofed to have a lower explanatory power compared to cDNA.
Increased Corg contents due to organic fertilization seem to provide excess electrons for all denitrification processes, mitigating the kinetically unfavorable process of N_2O reduction. These results emphasize the need for appropriate soil management to minimize N_2O emissions especially for soils with organic fertilization history.

The effect of soil enrichment by antagonistic strains on the severity of common scab of potatoes

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Common scab of potatoes is a disease that is difficult to manage due to complex interactions among the pathogenic bacteria, host plant genetics, soil and microbial community. Eighty five strains of actinobacteria were isolated from soil and surface of potatoes including the lesions of common scab. Strains were collected in eight fields under crop rotation in the Czech Republic in 2009, 2010, and 2014. Actinobacteria strains were assessed for their abilities to inhibit the growth of phytopathogenic strains *Streptomyces scabiei* (DSM 41658) and *S. acidiscabies* (DSM 41668) on agar plates by co-cultivation. Zones of inhibition of the pathogens were evaluated by a four degree scale three days after inoculation on pre-inoculated plates with tested antagonists (28°C, GYM medium). Also, the ability of fourteen strains showing strong antagonistic effect against one or both pathogens to grow in vermiculite was assessed. Inoculated vermiculite (2.4g of vermiculite plus 6.8ml of inocula in 100mL Erlenmeyer flasks) was cultivated for 21, 43 and 70 hours, 5 and 10 days (stationary, 28°C). Growth rate of strains was assessed by counting of CFU after serial dilutions plated on GYM agar plates.
Three individual strains and mixture of six selected strains were used for a pot experiment. The pots were filled with soil enriched with actinobacteria inocula in vermiculite to a final concentration of approx. 108 CFU/L of soil. Five replicates of each treatment were planted with the sensitive cultivar Agria. Potatoes were harvested 90 days after planting. Severity of common scab was evaluated using a nine point scale based on the percentage of surface covered by lesions. The treatment with strain 09Zd22 had significantly lower scab severity ($p = 0.001$) with the mean 3.3 in comparison to the mean of controls 4.7. The most effective strain in the pot experiment had the fastest growth rate in vermiculite while the most antagonistic strain with slow growth rate had no significant effect on scab severity.

Bead-beating and isolation of environmental nucleic acids

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Microorganisms represent fundamental and irreplaceable part of all ecosystems. They are responsible for nutrient cycling in nature and involved in nearly all important processes in terrestrial and aquatic ecosystems. They generate nutrients in bases of all food chains and their extensive metabolic potential is utilized in many areas of human activities. Further expansion of utilization of microbes or their products in agriculture, various biotechnological applications and other human activities depends mainly on increasing knowledge of their diversity in various ecosystems, mainly in soil, which represents immense source of microbial diversity. Various methods are used to approach and explore this diversity, majority of them being based on isolation of microbial nucleic acids. One of the first steps in all extraction methods is mechanical treatment of soil (and other) samples. Tone of the most widely used mechanical treatments these days is so-called bead-beating. This treatment serves physical disruption to disperse soil aggregates and – at the same time –starts the extraction process itself. It is performed using various instruments called bead-beater, ribolyser, MagNa Lyser etc, which all use vigorous shaking of samples in a buffer with various beads. It is surprising that not very big attention is payed to exact parameters of this treatment as it can utilize not only various types of beads, but can differ also in length and intensity of beating itself. We tested all these variables and their possible influence of the overall results of diversity analysis will be discussed in our presentation.

Effects of management on soil microorganism communities in Swiss vineyards

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Viticulture is an important agricultural sector in Switzerland that shows extremely intensive practices, leading to homogenous landscapes. The aim of the present study was to show the benefits of less intensive management practices on soil ecosystem functions in vineyards. We studied a disturbance gradient by selecting vineyards with different plant cover levels. We investigated soil microorganism communities by analysing phospholipid fatty acid (PLFA) profiles as well as potential links between these communities and diversity of other trophic level indicators such as ground beetles (predators) and plants (producers). We hypothesized that along our disturbance gradient, we will find a general increase of biodiversity with decreasing disturbance.

Alfalfa root symbionts under soil nutrient pressure: cooperation or competition?

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Introduction: Understanding the role of root symbionts for plant nutrients uptake is of great importance in agro-ecosystems as they can directly impact the availability of nutrients in soil as well as crop plant growth and productivity. However, still little is known on the interactions among root symbiotic partners in natural systems.
Objectives: In this study, we aimed to question whether the nitrogen fixing bacteria (i.e. rhizobia) were in competition with arbuscular mycorrhizal fungi (AMF) to form symbiotic interactions in the rhizosphere of *Medicago sativa* (Alfalfa) as well as to understand their response to long term nutrient inputs in agricultural soils.
Methods: Total DNA and RNA were extracted from rhizosphere soil of Alfalfa subjected to differential mineral (NPK) and farmyard manure (FYM) long term fertilization. High-throughput sequencing of bacterial and fungal SSU rRNA genes enabled a deep characterization of total and active communities of rhizobia and AMF OTUs, while a network analysis further provide insights in their co-occurrence and exclusion patterns.
Results: The presence and activity of AMF was undetectable in soil fertilized with both FYM and NPK suggesting a loss of mycorrhization due to high nutrient availability for the plant. On the contrary, total rhizobia OTUs richness and relative abundance positively correlated with the mineral fertilization treatment (one-way ANOVA, $p < 0.01$) while no significant variations were observed in rhizobia activity patterns. A network analysis further showed that AMF OTUs co-occurred more often than expected by chance with other AMF OTUs, probably reflecting their habitat preference for low nutrient soils. Although, DNA and RNA networks shared only 22 % of their co-occurrences and exclusions, they displayed a similar modular structure shaped by the fertilization treatments. Only three AMF – rhizobia OTUs co-occurring pairs were detected in the DNA network (*Diversispora – Burkholderia; Glomus – Aquamicrobium;* unidentified *Glomerales – Pedomicrobium*), while the majority of co-occurring pairs among symbionts were between rhizobia OTUs. No exclusion could be observed between symbionts OTUs in both networks, highlighting the absence of detectable competition between AMF and rhizobia in the studied soils.

Activation of salicylic acid defence signalling pathway reduced *Archaea* abundance and genes involved in nitrogen and carbon cycling in wheat rhizosphere

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Salicylic acid (SA) signalling is activated by plants to combat biotrophic pathogens. In the present study, we examined the effect of SA pathway activation on the composition of microbial communities as well as the abundance of genes for carbon and nitrogen cycling in wheat (*Triticum* spp.) rhizosphere. We tested the hypothesis that the activation of SA pathway in wheat alters the composition of microbial communities and the functional diversity in wheat rhizosphere. Wheat was grown in agricultural soil with continuous wheat cropping for 19 years. Exogenous application of SA was implemented on 10 day-old wheat seedlings, and rhizosphere soils were collected at 48 h and 72 h after SA application. Phylogenetic marker gene sequencing (16S rRNA gene) was used to characterise the diversity of bacterial and archaeal communities associated with wheat roots. Chitinase A and nitrogen cycling genes including *arch-amoA, amoA, nifH, nosZ, narG* were quantified to verify whether there are changes in functional diversity in the wheat rhizosphere. Exogenous SA application activated SA signalling, and did not directly influence microbial communities in bulk soil. Marginally significant changes in rhizosphere community composition were observed (P = 0.097, 48 h; P = 0.093, 72 h). Activation of the SA signalling increased two *Lysobacter* and *Pseudomonas* species that have been shown to be involved in biocontrol. However, activation of the SA signalling pathway triggered a significant decrease in archaea member *Candidatus Nitrososphaera* at both time points. In addition, the *amoA-arch* gene was also less abundant in rhizosphere samples from plants with activated SA pathway, as revealed by qPCR. Chitinase A decreased in abundance in rhizosphere soils at both 48 h and 72 h after SA treatment, while *nifH, amoA* and *nosZ* were significantly supressed at 72 h (P = 0.009, P = 0.003, P = 0.03). Our results suggest that SA signalling altered the wheat rhizosphere microbiome for plant defence and led to a decrease of bacterial components involved in carbon and nitrogen cycling.

Newly isolated denitrifiers from low and high pH soil show little correlation between genotype and phenotype

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We performed an isolation program of nitrate reducing/denitrifying/DNRA bacteria from soils sampled from two plots (pH 3.7 and pH 7.3) in a long-term liming. Soil solutions were spread on diluted tryptic soy agar buffered to pH 7.4 (both soils) or pH 5.7 (low pH soil) and incubated aerobically. Colonies were picked after one and two weeks. The isolates (182 in total) were inoculated into sealed serum vials containing 1/10 TSB, 1 mM NO^{3-} and 1 mM NO^{2-}; 1% O_2 and with 1% N_2O in headspace. End-point analyses of NO^{3-}, NO^{2-}, NO, N_2O and N_2 revealed that 11 of the isolates (6%) performed full-fledged denitrification (8 from the high- and 3 from the low-pH soil) while 6% lacked the last step (N_2O reduction to N_2), thus potentially contributing to N_2O emission. Twenty-one of these strains were whole genome sequenced (Illumina MiSeq). The isolation strategy proved to be successful in capturing organisms representing a wide phenotypic and taxonomic diversity. The isolates represented four phyla: Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. As expected, full-fledged denitrifiers were found mainly among the Proteobacteria. Among the low pH isolates, 8 belong to the genus *Rhodanobacter* within Gammaproteobacteria We analyzed the denitrification phenotypes of these *Rhodanobacter* strains in response to pH 5.7 and pH 7.3, using a robotized incubation system for determination of denitrification kinetics, which monitors O_2 and all gases relevant for denitrification (NO, N_2O and N_2). This was coupled with repeated liquid sampling for nitrite and OD measurements). The strains showed radically contrasting phenotypes during transition from oxic to anoxic respiration. Four out of the six strains carrying a type I *nosZ* gene, together with two strains lacking it, accumulated N_2O at both low and high pH but showed different maximum NO concentrations (0.6 µM-60 µM). Two strains were capable of reducing N_2O to N_2 at pH 5.7, while at pH 7.3 nitrite was recovered as N_2O at the end of the anoxic incubation. These strains were phenotypically identical with the type strain *R. denitrificans* 23569, which we also investigated. These findings show that there is no clear correlation between phylogeny and phenotypes even for closely related microorganisms. Moreover, the phenotypic outcome under a given condition (low vs high pH) could not be predicted from the sequence of the functional gene (*nosZ*).

The pontential of agroecosystem services in relation to land use and

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The concept of ecosystem services has become an important tool for modelling interactions between ecosystems and their external environment in terms of global bio-climatic changes. The provision of ecosystem services depends on biophysical conditions and changes over space and time due to human induced land cover and land use. Traditionally, agroecosystems have been considered primarily as sources of provisioning services, but more recently their contributions to other types of ecosystem services have been recognized. According to several authors agroecosystems can provide a range of other regulating and cultural services to human communities, in addition to provisioning services and services in support of provisioning. Six agricultural study areas, each of them with two different land use categories (arable land and permanent grasslands) located in various natural conditions of Slovakia, were evaluated. For the analysis of the agro-ecosystem services study sites were selected on the basis of the following criteria: 1/polluted area (inorganic contamination) , 2/non polluted area (without the inorganic contamination), 3/ area threatened by erosion, 4/ abandoned land, 5/ low productive land, 6/ productive land. The relationship among provisioning services, regulating services (filtration of the inorganic pollutants, carbon sequestration, potential of erosion- USLE model), cultural sevices (according SolVES model), soil abiotic parameters (pH in KCl, soil organic carbon - Cox, total nitrogen - Nt, nutrients - P, K, Mg), biodiversity (Shannon-Wiener index), macro-organisms (earthworms and earthworm fresh body biomass, earthworms were collected by hand sorting (35x35x20 cm of soil), micro-organism (according to MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) were evaluated. The greatest differences can be seen in the relation to sites land use and diversity of soil type, from the abiotic factors is that the soil pH value and P- nutrient conten and oxic conditions in the soils.

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Enzymes related to organic matter degradation and agricultural management

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Soil microorganisms play an important role in the health of the vegetation through nutrient mineralization and maintaining soil structure. They are known to be most diverse and complex, as there could be a billion microbes living in just gram of soil. This leads to poor understanding of the relation between microbial and functional diversity and also their influence over different ecosystem processes. Functional diversity of key microbial enzymes is important to understand complex ecosystem processes such as carbon cycling. Although NGS advancements have assisted us in understanding ecosystem processes by large extent, deducing enzyme diversity from metagenomes could still be tricky due to low coverage. We developed a targeted metagenomics approach primarily designed for metagenomics of functional enzymes in large-scale based on “sequence capture”. As a proof of concept in metagenomics, we have investigated enzymes in large-scale related to carbon degradation in agricultural wheat soil and a grassland soil. This particular large-scale implementation of sequence capture in metagenomics is reported for the first time. We targeted around 400,000 enzymatic sequences by creating probes primarily from their conserved regions. The fraction of targeted enzymatic sequences obtained from the datasets were about 40% compared to 1.3% and 5.3% from untargeted and intensively sequenced publicly available soil metagenomes. This sequence capture method has been implemented on five pairs of agricultural and grassland soils from five different sites to analyse the distribution of our target functional enzymes over location and management. The conventional ribosomal (16S) sequencing has been compared to understand the link between the microbial and functional diversity and also their influence on different agricultural managements. Their expressions at different conditions (mRNA) were also tested using the same technique. Implementations of such inter-disciplinary techniques to ecosystem samples are key to their understanding and sustainability.

Changes in the oxidation-reduction potential and in bacterial profiles in the soil around direct-seeded rice under submerged conditions

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Among the systems for cultivating rice (*Oryza sativa* L.), the direct seeding method is becoming more popular than the seedling transplant method?because the former requires less labor and time. However, this cultivation technique has seen limited use in Japan due to poor seedling emergence and establishment. The poor seedling emergence and establishment of rice plants is typically attributed to a drastic decrease in the soil oxidation-reduction potential (ORP) around the direct-seeded rice under submerged conditions. However, data on the environmental changes of the soil around the direct-seeded rice under submerged conditions are lacking. The objectives of this study were to (1) assess the seedling emergence rate under different submerged soil conditions and (2) characterize the changes in the ORP and the bacterial profiles around the direct-seeded rice under submerged conditions.

The seedling emergence rate was 90% for 10 d when rice seeds were directly sown on the surface of submerged soils. In contrast, when the rice seeds were directly sown at a depth of 1 cm under different submerged soil conditions (sterilized, unsterilized and straw application soil), the seedling emergence rates were approximately 70%, 60% and 10%, respectively. The ORP of submerged soils with rice seeds drastically decreased compared with that of submerged soils without rice seeds. In the case of the straw application into the soil, the ORP of the submerged soil around direct-seeded rice drastically decreased within 1 d. These results suggest that the cause of the poor seedling emergence of direct-seeded rice under submerged conditions can be attributed to a drastic reduction of the ORP around the rice seeds, especially with the application of organic matter such as straw. Next, we examined the bacterial profiles around the direct-seeded rice using a submerged soil collection method and an Illumina MiSeq Next Generation Sequencer. Immediately after the submersion treatment, we detected aerobic bacteria such as *Bacillus* in the submerged soil around the direct-seeded rice. After 4 d, we detected anaerobic bacteria such as *Symbiobacterium, Geobacter*, and *Oxalobacteraceae*. The application of straw in submerged soils resulted in a significant increase in the proportion of *Clostridium*. Thus, we conclude that the causes of poor seedling emergence are partly due to a specific microbial activity and microbial metabolites that increased in the soil around the direct-seeded rice.

Biochemically contrasting organic inputs combined with mineral nitrogen fertilizer shape the temporal variation of ammonia-oxidizing prokaryotic communities in an agricultural soil

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Temporal variation of ammonia-oxidizing bacterial (AOB) and archaeal (AOA) *amoA* gene abundance was assayed in a tropical, agricultural Humic Nitisol during two cropping seasons of a field experiment in the central highlands of Kenya. Since 2002, soils were treated yearly with biochemically different quality organic inputs (4 Mg C ha-1 year-1) of *Tithonia diversifolia* (TD; C/N ratio: 13, Lignin: 8.9 %; Polyphenols: 1.7 %), *Calliandra calothyrsus* (CC; 13; 13; 9.4) and *Zea mays* (ZM; 59; 5.4; 1.2) combined with and without 120 kg $CaNH_4NO_3$ ha-1 season-1. Soils (0-15 cm) were sampled before incorporation of inputs in 2012 to reveal long-term effects, as well as in 2012 and 2013 at young growth (EC30) and flowering (EC60) stages of maize to assay short-term effects of contrasting inputs. Soils were subjected to DNA-based quantification (quantitative qPCR), and community composition analysis (T-RFLP) of the *amoA* gene analyses. Generally, AOB and AOA responded differently to organic and mineral nitrogen (N) inputs. In comparison to intermediate quality organic inputs (CC), high quality organic inputs (TD) promoted the abundance of AOB in the long and short-term, but only in the long-term for AOA. Low quality inputs (ZM) revealed contrasting effects on abundance of microbial communities which were specific to crop growth stage and microbial group. These findings were matched by those of AOB and AOA community composition. We attributed these findings to high lignin and polyphenol contents in CC compared to TD and ZM, promoting the formation of polyphenol-protein complexes limiting microbial communities from organic N access at the early stage of decomposition. Decrease of AOB abundance posed by ZM inputs in the long-term was attributed to its low N content. Besides, sole use of mineral N or its combination with organic inputs decreased AOA abundance but not AOB. This was ascribed to soil pH reduction in consequence of long-term mineral N use. Overall, observed input-related effects on soil prokaryotic communities were partially masked by contrasting climatic conditions during the two study seasons. We therefore recommend a prolonged study period to conclusively understand the noted resource-dependent niche differentiation between AOB and AOA. This approach should consider rRNA-based analyses to further understand the critical short-term dynamics of active nitrifying communities regulating nutrient release during cropping seasons.

Soil type, season and crop growth stage exert a stronger effect on rhizosphere microbial dynamics than the fungal biocontrol agent *Fusarium oxysporum* f.sp. *strigae*

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Striga hermonthica causes severe yield reduction in cereal crop production in Sub-Saharan Africa. Biocontrol agents (BCA) such as *Fusarium oxysporum* f.sp. *strigae* ("Foxy-2") were proven as effective and environmental friendly control strategies. By now, potential non-target effects of "Foxy-2" on beneficial indigenous microbial rhizosphere communities of affected crops has not been investigated under field conditions which is, however, a prerequisite for commercial use of the BCA. Hence, we hypothesized that, although inoculated "Foxy-2" may pose a considerable nutrient resource competition to indigenous rhizosphere microbial communities, its overall impact is minor relative to those of soil type, season and crop growth stages. Our objectives were (i) to monitor the abundance of "Foxy-2", and (ii) to investigate the abundance (quantitative PCR) and community structure (T-RFLP analysis) of indigenous total fungi as well as total and nitrifying (archaea (AOA), bacteria (AOB)) prokaryotes colonizing the rhizosphere of maize which was used as test crop. Rhizosphere samples were obtained at three growth stages (i.e.,early leaf development (EC30), flowering (EC60) and senescence (EC90)) of maize cultivated in two cropping seasons at two contrasting agro-ecological sites in Western Kenya. A treatment with N-rich *Tithonia diversifolia* residues was included to compensate a potential resource competition. It was observed that the abundance of "Foxy-2" was affected by crop growth stage, seasonality and soil type ($P<0.05$). Abundance of total bacteria, archaea, AOA and AOB was influenced by crop growth stage, seasonality and soil type, but not by "Foxy-2" inoculation ($P<0.05$). On the contrary, "Foxy-2" revealed a transient suppressive effect on total fungal abundance which was neutralized by organic amendment. Nitrifying prokaryotes and total fungal community structure was influenced by growth stage ($P<0.05$), but not "Foxy-2". It was concluded that organic resource availability, seasonal variations, crop growth stages and soil type shape the abundance of "Foxy-2", while the impact of "Foxy-2" on the abundance and community structure of indigenous rhizosphere microbial communities was negligible relative to the assayed influence factors. Hence, under the given environmental conditions, "Foxy-2" had to be regarded as an environmentally safe BCA.

Fumigation with Dazomet modifies soil bacterial and fungal communities in soil of apple orchards affected by Specific Replant Disease

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Specific Apple Replant Disease (SARD) is a disorder that affects apple trees when they are re-planted in soil where the same species was previously grown. Although it is long-time known problem, the specific causes are still poorly understood. Because soil fumigation commonly relieves, at least temporarily, the symptoms, one of the main hypotheses is that microorganisms play an important role, although SARD is most probably the outcome of a more complex interplay between abiotic and biotic factors. Until last decade, unravelling the complex dynamics of soil microbial communities was almost impossible, because only a small fraction of microorganisms is cultivable in vitro. The recent advances in culture-independent technologies opened new opportunities to improve our knowledge on the role of microorganisms in SARD. The aim of this study was to uncover the complex dynamics of microbial communities in SARD affected soils in apple orchards especially focusing on the differences in taxa abundances between fumigated (F) and non-fumigated soils (NF) by using NGS technologies. Soil samples were collected in both fumigated (Basamid; a.i. Dazomet 99%) and non-fumigated sites in SARD affected apple orchards from an area in the Trentino-Alto Adige region (Italy) having the same climate and soil characteristics. Because we were interested in the long term changes, sampling was carried out 18 months after fumigation, at the end of the second growing season. Total DNA was extracted and the fungal ITS and bacterial 16S regions were pyrosequenced with the Roche's 454 Platform. Both bacterial and fungal communities significantly differed between fumigated and control soils (PERMANOVA P=0.036 and P=0.042, respectively). *Bacillus sp., Pseudomonas sp., Streptomyces sp. and Cheatomium* sp. are the taxa identified by LEfSe as the most discriminative microbial biomarkers of fumigated soils (abundances: 1.5%±0.4% in NF vs. 2.7%±0.4% in F; 0.5%±0.2% in NF vs. 1.6%±0.4% in F; 0.3%±0.1% in NF vs. 0.8%±0.1%; 0,2%±0.1% in NF vs. 3.1%±1.9%, respectively), where apple trees thrived compared to the ones in untreated soils (non-fumigated). Our results suggest that, although a cause-effect relation with SARD cannot be proven, fumigation with Dazomet reduces SARD symptoms and modifies soil microbial communities, enhancing the presence of some beneficial microorganisms, known for their action against plant pathogens.

Improved general plant pathogen suppressiveness by agricultural management practices

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In winter, the crop cover of field is an effective mean for water protection in agriculture adapting to climate change. In the agricultural area of annual crops, the essential tools in order to increase the crop cover outside the growing season are direct sowing and minimum tillage. They may, however, favor plant diseases transmitted through soil and plant residues, and to increase the use of pesticides.
The overall project objective was to develop innovative agro-environmental technology appropriate for practice in arable farming, which reduces the need for chemical control of plant diseases, and increases the crop cover of arable fields outside the growing season for farming of annual crops. The project examined the impact of autumn tillage method (zero tillage, stubble cultivation, plowing) and crop rotation (continuous spring barley (*Hordeum vulgare*) vs. barley-faba bean (*Vicia faba*) –oats (*Avena sativa*)-rapeseed (*Brassica napus*) -rotation) especially on the occurrence of the soil and plant residue transmitted plant diseases, as well as on the development of the general plant pathogen suppressiveness in soil (measured as soil fungistasis). The project focused on finding solutions for the management of plant diseases (particularly pathogenic *Fusarium* fungi; test species *F. culmorum*), for which the pesticides available have a low response. The soil physical conditions (temperature, moisture) were measured continuously in the fields during and out of the growing season.
Based on the results, cultivation methods can have an impact on the general plant disease suppressiveness in arable soil. Reduced tillage improved the suppressiveness compared with plowing. The improved disease suppressiveness was related to the increase of the soil microbial biomass. In the treatments where the disease suppressiveness was the highest, the frequency of the pathogenic test fungus was the lowest.

Long-term nitrogen fertilization affects microbial communities regulating N_2O emissions in arable soils

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Arable soils are a major source of the greenhouse gas N_2O, and the only known sink in Earth's biosphere is the microbial reduction of N_2O to N_2 via the N_2O reductase. This enzyme is found among denitrifiers, however non-denitrifying bacteria may also have this trait. Nitrogen fertilization generally increases both denitrification and N_2O emissions, but also changes the relative abundance of certain microbial taxa. Based on genome studies of the co-occurrence of denitrification genes, we hypothesize that these directional changes in the community ultimately determine a soil's N_2O:N_2 emission ratio. To test this, the impact of N-fertilization was determined using 14 geographically diverse Swedish long-term field trials, in which fertilized and non-fertilized soils were compared. Quantification of genes involved in nitrite (*nir* genes) and N_2O reduction (*nosZ* clade I and II genes) showed no general trends in relation to N fertilization. Furthermore, the abundance of *nosZI*, mainly found among denitrifiers with a complete pathway, was higher than *nosZII*, common in non-denitrifying N_2O reducers, across all sites, suggesting that denitrification is an important N_2O reduction pathway in arable soils. Low *nir:nosZ* ratios revealed a dominance of organisms producing N_2O compared to those reducing it, irrespective of fertilization. To resolve the underlying ecological processes structuring the total bacterial and N_2O reducing communities in relation to edaphic factors and fertilization, the 16S rRNA and *nosZ* genes were sequenced. The community data will be compared to N_2O:N_2 ratios to further understand the importance of community membership for N_2O reduction capacity.

Impact of strategic tillage on nitrogen cycle genes (*amoA* and *nifH*) in no-till systems in Queensland, Australia

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The adoption of no-tillage (NT) farming systems has improved soil quality and crop profitability in Australia. However, this practice has increased incidence of herbicide-resistant weeds, soil stubble-borne diseases and stratification of nutrients in the surface soil. To control these issues, an occasional strategic tillage (ST) has been increasingly utilised during the fallow period in the Northern grains region of Australia. ST has been defined as an occasional tillage that takes into account soil water content, tillage frequency, timing as well as type of implements. Disturbance in NT has the potential to influence soil quality and microbial communities affecting directly or indirectly soil nutrients cycles lincluding nitrogen. Investigating the abundance of genes involved in the nitrogen cycle genes and consequently the changes that a ST may induce will add information that is highly relevant to the management decision. In this study, we assessed the impact of an ST on the abundance of nitrogen cycle genes (*amoA* and *nifH*) involved in autotrophic ammonia oxidation as indicators of soil health after disturbance. The experimental sites included three locations in the Australia's northern grains region (Biloela, Jimbour and Hermitage) with 5 to 46 years of continuous NT. Tillage treatment for Biloela and Jimbour sites included operations of a single tillage (December 2012 or January 2013 or March 2013), dual tillage (December 2012 and January 2013) and triple tillage (December 2012, January 2013 and March 2013). Tillage was performed using a chisel plow (Biloela), narrow chisel point and offset disc (Jimbour) to depths between 0 to 0.15 m. At Hermitage site, the original treatments of NT and conventional tillage which are in place for 46 years were split longitudinally in two. Half received chisel tillage in March 2012 and the other half was left untilled. Seven soil samples were collected from the 0.0-0.1 m and 0.1-0.2 m soil profiles in April (Biloela and Jimbour) and May (Hermitage) 2013 and composited according to each depth. Gene abundances were measured by utilising quantitative real-time polymerase chain reaction (qPCR). Given that soil physicochemical properties were previously shown to be almost unaffected by ST at all sites, we tested the hypothesis that ST has minor impacts on the abundance of the selected nitrogen cycle genes.

Isolation of a novel ammonia oxidising archaeon, representative of the *Nitrososphaera* 'sister' lineage

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Nitrification links the most reduced form of nitrogen, ammonium, to the most oxidised form nitrate, and is a fundamental part of the nitrogen cycle. In aerobic environments, the first step in this process was thought to be mainly performed by ammonia oxidising bacteria (AOB) within the beta- and gammaproteobacteria. However, the discovery and cultivation of a marine, chemolithotrophic ammonia oxidising archaeon (AOA) in 2005 dramatically changed our understanding of which organisms are contributing to this process. Five major lineages of AOA have now been identified; the *Nitrosopumilus, Nitrosotalea, Nitrosocaldus, Nitrososphaera* and *Nitrososphaera* 'sister' lineage. Only six AOA had been isolated in pure culture, with each of the five lineages represented except the *Nitrososphaera* 'sister'-lineage. The first isolated representative of this lineage was obtained in this study, and appears to have similar characteristics to other, neutrophilic, chemolithotrophic AOA. However, it appears to have a surprising tolerance to high concentrations of ammonia unlike other previously cultivated AOA.

Annotation of gene cluster Involved phenazine biosynthesis in *Streptomyces* CMAA 1322 also too structural elucidation of 1,6 dimethoxyphenazine.

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Complex polyketides are microbial natural products whose chemical structures correlate with the sequence of the modular enzymes responsible for their biosynthesis. Now, genome-scale sequencing of actinomycetes has revealed untapped chemical diversity in culturable *Streptomycetes*: there are many biosynthetic clusters present but most are cryptic (silent). Expression of biosynthetic genes is now a severe bottleneck in trying to develop a genuine synthetic biology of natural products, in which PKSs are designed and fabricated to order to produce a specificed target molecule. Phenazines are versatile secondary metabolites of bacterial origin that function in biological control of plant pathogens and contribute to the ecological fitness and pathogenicity of the producing strain. The model lineage used in this study is *Streptomyces* sp. CMAA 1322, was previously isolated from soils of the Caatinga biome located in semiarid northeastern Brazil (S09°27'8" W43°23'48,3") and according to preliminary studies of the analysis of the genome of strain model is a new species. Genome scanning identified 10 secondary metabolism, contains a complex phenazine biosynthetic pathway (phzA1B1C1D1E1F1G1), coding for biosynthesis of a wide of structural types. Bioprospect to the crude extract by spectrometric methods isolate and elucidate the an antifungal compound synthesized by phenazine clusters, 1,6 dimethil-pehazine. This work exemplifies not only the discovery of rare antibiotics produced by *Streptomyces*, but also the utility of genomics as a further tool, complementary to spectroscopy, to enable rapid determination of complex structures.

Soil oomycete community structure association with cavity spot disease of carrot

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Cavity spot in carrot is one of the major problems in carrot cultivation especially in the temperate part of the world. The disease is caused by a complex of *Pythium* species. The genus *Pythium* belongs to the oomycetes. Culture independent studies have opened new possibilities to study disease complexes including those caused by oomycetes. In this study, we examined oomycete communities in symptomatic plant tissue and the adjacent soil using cavity spot disease in carrot as model system. We sampled i) soils from fields intended for carrot production, ii) soil from fields where carrot was already growing and iii) cavity spot symptomatic tissue. High throughput sequencing of the samples targeting the ITS1 region of ribosomal DNA from oomycetes yielded 342,972 reads after quality control resulting in 111 operational taxonomic units excluding singletons. After assigning taxonomy, we found that Phythiales dominated the reads revealing a huge diversity of *Pythium* species including both pathogenic and saprophytic species. The dominant *Pythium* species in 60 soil samples before carrot cultivation were *P. attrantheridium, P. apiculatum, P. heterothallicum, P. sylvaticum* and *P. irregulare*. However, in the presence of carrot, the top 5 dominant species in the soil samples were *P. irregulare, P. attrantheridium, P. heterothalicum, P. sylvaticum* and *P. apiculatim.* Indicator species analysis showed that *P. irregulare, P. heterothallicum, P. sylvaticum, P. apiculatum,* and *P. intermedium* were more abundant when carrot was present in the field. Dominance of *Pythium* species was also observed in the cavity spot lesions, and most of the species found were plant pathogenic species including *P. violae* and *P. sulcatum*.

Evaluation of Effectiveness of Rhizobia and Plant Interaction in Different Soil Types

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Leguminous plants have a high nutritious value, therefore promotion of legume use in agriculture can be observed. It is known that legume features are improved because of their ability to fix nitrogen from the atmosphere, due to their symbiotic relationship with rhizobia bacteria. The symbiotic relationship between legumes and rhizobia benefits also the next crops, as the nitrogen level in soil can be enhanced, in this way eliminating the need for chemical fertilizers. It is important to remember, that the yield increase is not always the main factor, when looking at the crop productivity. Improvement of food and feed quality over quantity should be taken into account more, especially in developed countries. As protein is an integral part of food and fodder, we should look at the protein content in legumes and the possibilities to increase it by using biological agents like rhizobia, depending on the genotype of rhizobium strain used for inoculation.
Pilot experiments were carried out in order to examine the possible impact of rhizobium strain genotype, soil and the choice of plant cultivar on the protein content of broad beans and peas. The genetic relatedness between different rhizobia strains was determined based on partial 16S rDNA sequences of different rhizobia strains. Protein content in different cultivars of pea and faba bean seeds was determined using mature, dry seeds. These experiments were conducted in different types of soil to observe the impact of the soil conditions on the protein content of beans and peas.
Overall, the protein content increase was observed after seed inoculation with rhizobia. Strain and plant cultivar interaction specification was observed. The effect of rhizobia inoculation on the content of protein was dependent on the rhizobia strain used. Plant cultivar also appeared to have a decisive role in protein content formation. These pilot experiments suggest that indeed rhizobia strains with different genotype show different protein content. However, other factors such as soil type and the plant cultivar also have an impact on the content of protein in broad beans.
Experiments were carried out at the Latvia University of Agriculture, Institute of Soil and Plant Sciences. This research is supported by the 7th Research Framework Programme of the European Union project 613781, EUROLEGUME (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed).

Microbial activity along a continuous subsurface core from an agriculture field at the estuarine region of the Mahi river: correlation with sediment characteristics

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Soil microbial activities play important role in biogeochemical cycling, soil formation, organic matter decomposition and are indicators of soil fertility and soil health. The broad estuarine mouth of the Mahi River is characterized by complex geomorphic setting including estuarine sediments bounded by vertical alluvial cliffs comprising late Pleistocene sediments that extend up to the coastline. The geomorphic evolution of the estuary is presumed to have been controlled by tectonic uplift and sea level changes during the Holocene. Agricultural fields within estuarine zone of Mahi river are made up of sediments of middle to late Holocene forming a younger terraced surface. The present study was aimed at understanding the microbial activity in the estuarine sediments in relation to physicochemical characteristics in core samples up to ~7.5 m depth recovered by drilling process.
Soil enzymes activity determination showed that there was significant activity in deeper samples. Interestingly some of the deep samples shows higher enzyme activity e.g. CRD 16 showed highest acidic phosphatase activity (i.e 138.013 ug pNP g^{-1} $2h^{-1}$ at ~ 6.6 m depth), CRD 21 shows highest alkaline phosphatase activity (i.e 105.35 ug pNP g^{-1} $2h^{-1}$ at ~7.25 m depth) and CRD 9 (~3.25 m depth) shows 30.04 ug pNP g^{-1} $2h^{-1}$ β-glucosidase activity. Total viable heterotrophic bacterial count was enumerated with Reasoner's 2A (R2A) agar. Significant numbers of culturable heterotrophes were obtained at various depths within these sediment samples. The ratios of fast growers (r-strategists or copiotrophs) to slow growers (k-strategists or oligotrophs) microbes was highest in samples located at 1.28 m depth and 3.28 m depth that is 0.316 and 0.352 respectively. The correlation of microbial parameters with sediment characteristics showed enzyme activities to be correlated with each other but not with moisture content or organic carbon. Specific counts of organisms that affect plant growth such as N_2 fixers, denitrifiers and cultural independent bacterial diversity determined by DGGE will be presented.

Diversity and spatial distribution of diazotrophs associated with micro-environments of wetland rice

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Rice is one of the world's most important crop plants. The production is strongly limited by nitrogen (N) availability, which is typically supplied by industrial fertilizers that are costly and hazardous to the environment. It is known that Biological Nitrogen Fixation through N_2-fixing bacteria and archaea (diazotrophs) can alleviate the N-shortage in rice cultivation. However, our knowledge on the micro-sites of N_2 fixation, as well as the diversity and in situ N_2 fixation activity of diazotrophs in the soil-microbe-plant interface (i.e. rhizosphere) of flooded rice fields is still rudimentary.

A greenhouse study was performed to identify key factors that control rice-diazotroph association and related N_2 fixation activities. Paddy soils of different geographical origin were cultivated with two genotypes of wetland rice. Samples were taken from the bulk soil, the root-associated rhizosphere soil, the rhizoplane, and the endosphere at flowering stage of rice plant development. These samples were subjected to functional assays and identification of the inhabiting diazotroph communities.

Based on Illumina sequencing of 16S rRNA and *nifH* genes and transcripts, we will present data on the diversity of diazotroph communities with emphasis on assessing the potential influence of (a) the soil microbial "seed bank" or (b) the plant activity in shaping the microbiomes in each micro-environment. Potential N_2 fixation activities of each soil-genotype combination and micro-environment will be shown on the basis of incubation assays using $^{15}N_2$-containing atmospheres. The localization of selected diazotrophs on the rhizoplane of rice roots via fluorescence in situ hybridization and confocal laser scanning microscopy will help to identify areas of potential N-transfer between diazotrophs and rice roots.

Methanotroph diversity increases methane oxidation

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Biodiversity increases ecosystem functions and stabilises ecosystem processes through time. These relationships have been extensively studied in plant communities. Less is known about the importance of the diversity of microbial communities for the soil ecosystem functioning, despite the paramount importance of these processes for global biogeochemical cycling.
We tested effects of biodiversity on ecosystem function in a specialized group of soil bacteria, the methanotrophs. These organisms are important for the global greenhouse gas budget. Most Methanotrophs use methane-oxidation as their only source of carbon and energy and are found in a variety of environments such as rice paddies, wetlands and landfills.
Using pure strains of methanotrophic bacteria, we assembled artificial communities containing one, two, four, six and ten strains. These communities were grown in microcosms for 44 days. Methane and CO_2 concentrations were determined every two days. We sequenced the 16S rRNA and *pmoA* genes from community samples collected after 0, 16 and 44 days.
Sequencing demonstrated that, while after 44 days some strains were more dominant and others rarer, the established diversity gradient was maintained. Net methane consumption and CO_2 production increased statistically significantly with higher strain richness in some phases of the experiment (days 8 to 22).
This study is one of the first to show a significant biodiversity – ecosystem function relationship in microbial communities. This demonstrates that biodiversity – ecosystem function relationships show similar patterns across scales and kingdoms and are therefore likely universal. Additionally, biodiversity is very likely also important in natural methanotrophic communities for the ecosystem function “methane oxidation”.

Roots of decline? – The SARISA project

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In modern agriculture, farmers often use only a few economically viable crops resulting in shorter rotations of two to three years. Traditionally, oilseed rape (OSR) was grown in a five year rotation mostly with cereals. The cropping area for OSR in the UK has increased over the last years as a direct responds to increased demand for protein meal used in animal feed and for vegetable oil for biodiesel and human consumption. This has resulted in high prices for OSR and therefore an additional pressure for shorter crop rotations. But this agricultural practice often comes along with static or even decreasing yields. High inputs of fertilisers and herbicides can partially compensate for shorter rotations, however the yield gap between 1st OSR sowings and short rotations has increased. Traditionally, phosphate and potash fertilisers were applied to OSR but the demand is decline. There is a growing body of evidence that insufficient use of sulphur fertiliser may be a limiting factor to yield. Symptoms of sulphur deficiency were yellowing of young or middle leaves, pale yellow flowers and can lead to noticeably reduced yield. Until now the complex interactions and their impact on yield decline between soil characteristics, the resultant microbial community composition in bulk and rhizosphere soil and the influence of the plant under different rotations were largely unknown. For this purpose soils from OSR trials with different rotations and soil types were analysed. Furthermore Illumina sequencing of 16S/18S rRNA gene and ITS fragments were used to determine the microbial community composition in bulk and rhizosphere soil. With this cultivation independent method it will be possible to identify the core microbial community associated with healthy soils and any additional groups not normally associated such as pathogens which are directly linked to OSR yield decline. Shorter rotations could increase the abundance of pathogens towards a critical level as already predicted by previous studies. In contrast to pathogens, many bacteria are known for positive effects on plants. It is possible that beneficial microbial groups are reduced in abundance by shorter rotations. Results of this project will help to give concrete practical advice to farmers by generating baseline data regarding microbial community composition under diverse conditions and revealing interactions between plant and environment.

Microbial diversity and ecosystem functioning in vineyards

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The BiodivERsA/FACCE-JPI project PromESSinG (www.promessing.eu) investigates the links between management, soil biodiversity and ecosystem functions in vineyard ecosystems in five European countries (CH, F, D, A, ROM). In standardized experiments replicated in each country, we investigate how different levels of plant cover affect the soil microbial community structure and function. Here, we present results from the first study year (2015) obtained in Switzerland. In particular, we study how soil management practices affect soil respiration and decomposition rates, microbial biomass, and genetic diversity of soil microorganisms. Furthermore we relate the results to different indicators of grape quality. The ultimate aim of this study is to advise on soil management to maintain high levels of grape quality while conserving microbial soil diversity and functioning in vineyard ecosystems.

Microorganism's enzymes implication in nitrous oxide emissions in a natural agricultural field at a fine time scale study

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Among the main greenhouse gases (GHG), nitrous oxide (N_2O) causes a serious environmental problem because of its global warming potential which is 298 times higher than CO_2 and because of its lifetime of 114 years. It is well known that microorganisms play an essential role in N_2O emissions and that agricultural soils emit most of this GHG. Recent studies highlighted the underestimated role of archaeal and fungal population in N_2O emissions and the importance of their involvement in this process. Thus, understanding which microorganisms contribute mainly to N_2O emissions and the study of their ecology is of great interest to anticipate N_2O emissions and improve good agricultural practices recommendations.
The objective of the current work is to evaluate at a molecular level the involvement of global microbial community in the N_2O release during a N_2O peak production at a fine time scale (hours). Indeed, N_2O production occurs mainly as peak emissions which lasts 24 to 48h, depending of climatic parameters. Thus, N_2O flux was measured before (background level) during and after a N_2O peak event (up to 150 ng N_2O.N m-2 s-1) with automatic chambers at an agricultural field (Gembloux, Belgium) after a natural rainfall.
Each day, total RNA was extracted from core soil samples in order to get information during the entire experience. The microbial community structure (bacteria, fungi, archaea) was followed by DGGE analysis and the expression of specific genes (*nirK, nirS, nosZ, cnorB*) encoding for enzymes involved in the nitrogen cycle has been assessed by quantitative PCR. Results will be presented on the poster.

Exploring the phytopathogenic seedbank of agricultural soils: diversity of soil borne plant pathogens in relation to edaphic properties and the soil microbial community

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As part of the total soil microbial community, soils harbor a reservoir of plant pathogenic fungal and oomycetal propagules surviving periods without the presence of a suitable host crop. This so-called phytopathogenic seedbank, is a reservoir for future outbreaks of crop diseases. Next generation sequencing offers an unprecedented way to zoom in on the composition of this pathogenic seedbank. Our aim was to obtain insight in environmental factors that drive the composition of fungal and oomycetal plant pathogens present in soil. To this end, we assessed the alpha- and beta diversity of potential plant pathogenic fungi and oomycetes for 50 agricultural soils in the Netherlands and determined relationships with physico-chemical soil properties, crop and management history, spatial patterns and non-pathogenic microbial community composition. Our results indicate that the composition of pathogen propagules in soil is driven by pH, soil type, crop history, spatial patterns and various microbial groups, e.g. the relative amount of litter saprophytes. The major driving factors differed for fungal and oomycetal pathogens as well for root- and shoot-infecting pathogens, suggesting interactions between environmental factors and pathogen traits like reproduction, survival and dispersal. This information appears to be of basic importance to identify risks for disease outbreaks and to suggest management strategies to prevent such outbreaks.

Effects of black pepper-vanilla rotation on vanilla rhizosphere fungal communities in relation to Fusarium wilt disease

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In this study, black pepper-vanilla rotation was found to be an effective method of reducing the vanilla soil-borne Fusarium wilt disease. Using Illumina high-throughput sequencing of the internal transcribed spacer (ITS) region, the soil fungal communities of bulk soil and rhizosphere soil in black pepper-vanilla rotation and vanilla continuous cropping system were compared. Black pepper-vanilla rotation resulted in a significantly higher fungal diversity than vanilla continuous cropping system. UniFrac-weighted PCoA analysis showed significant variations in bulk soil of fungal community structures between black pepper-vanilla rotation and vanilla continuous cropping system, and suggested fungal community structures were seriously affected by the vanilla root system. Moreover, black pepper-vanilla rotation system significantly reduced the Fusarium abundance in vanilla rhizosphere soil and increased the putatively plant-beneficial fungal groups, such as *Trichoderma* and *Penicillium* genus, which might explain for the decrease of vanilla Fusarium wilt disease in black pepper-vanilla rotation system.

Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health

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Host-associated bacterial communities can function as an important line of defense against pathogens in animals and plants. Empirical evidence and theoretical predictions suggest that species-rich communities are more resistant to pathogen invasions. Yet, the underlying mechanisms are unclear. Here, we experimentally test how the underlying resource competition networks of resident bacterial communities affect invasion resistance to the plant pathogen Ralstonia solanacearum in microcosms and in tomato plant rhizosphere. We find that bipartite resource competition networks are better predictors of invasion resistance compared to resident community diversity. Specifically, communities with a combination of stabilizing configurations (low nestedness and high connectance), and a clear niche overlap with the pathogen, reduce pathogen invasion success, constrain pathogen growth within invaded communities and have lower levels of diseased plants in greenhouse experiments. Bacterial resource competition network characteristics can thus be important in explaining positive diversity-invasion resistance relationships in bacterial rhizosphere communities.

The functional profiles of soil microbial communities are determined by soil chemical properties but not community composition

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Soil microbes are the most important driver for the biogeobiochemical processes, and a particular microbial group is usually associated with some unique functions. The functional profiles of microbial community may be determined by community composition. On the other hand, soil chemical properties are the main environmental cues shaping the soil microbial community. However, the effects of soil chemical properties and microbial community on the functional profiles are far from understanding. In this study, we constructed special rhizoboxes with root chamber (RC) and rhizosphere space, in order that the rhizosphere soils with different distances from RC could be easily sampled. Soils in RC and in the near (Rn, 0-5 mm from RC) or the far rhizosphere (Rf, 10-15 mm from RC) were sampled. Soil chemical properties were measured. Microbial community composition was monitored with PLFA as the biomarkers. The functional profiles of microbial community were evaluated using the soil enzyme activities (SEA) and the quantification of functional genes.
Results showed that the contents of TOC and DOC were similar in RC, Rn and Rf. Available N content was lower in RC than in Rn or Rf for the gaminoid species but kept unchanged for the legume species. Available P content was similar in RC, Rn and Rf, while available K content was much different in the following order: Rf > Rn > RC. PLFA analysis showed that the microbial community was more diverse in RC than in Rn or Rf. Moreover, PCA indicated that the microbial community composition in RC was much different from that in Rn and Rf, with the latter similar to each other. SEA assay revealed that the activities of cellulose, cellobiosidase, β-glucosidase, nitrate reductase, leucine-aminopeptidase were not affected, however, the activities of β-glucosidase, β-xylosidase, decreased in Rn and Rf compared to RC. qRT-PCR of functional genes showed that the abundances of AOB-*amoA, nirS, nifH* and *alp* were affected. To demonstrate the relationship between functional profiles of microbial community and soil chemical properties or microbial community composition, Pearson correlation (by Mantel test) analysis was conducted with R=0.261 (P=0.010) for soil chemical properties and -0.058 (P=0.420) for microbial community composition. This suggests that the functional profiles of soil microbial communities are determined more possibly by soil chemical properties than by microbial community composition.

The biocontrol agent *Fusarium oxysporum* f.sp. *strigae* – its detection and effects on beneficial indigenous microorganisms in a maize rhizosphere

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Integrating resistant crop varieties and *Fusarium oxysporum* f.sp. *strigae* (Fos) as biocontrol agents (BCAs) was shown to be effective in controlling the parasitic weed *Striga hermonthica* which acts in the rhizosphere of several tropical cereals. Persistence of Fos inoculated to soils and its effects on beneficial indigenous microbial rhizosphere communities has however not been observed by now, although it is a prerequisite for prospective field application. Hence, our objectives were (1) to develop an explicit AFLP-marker to quantify the abundance of Fos, and (2) to assess the potential impact of Fos on indigenous nitrifying prokaryotes and arbuscular myccorrhizal fungi (AMF) in a maize rhizosphere cultivated on two distinct tropical soils (sandy Ferric Alisol versus clayey Humic Nitisol). We applied the known Fos-BCA "Foxy-2" as model organism via seed coating of a *S. hermonthica* tolerant maize variety to the two soils. Proliferation of "Foxy-2", nitrifying prokaryotes and AMF was followed at 14, 28 and 42 days after experiment start considering two critical influence factors: (1) presence of *S. hermonthica* plants, and (2) application of *Tithonia diversifolia* residues to compensate a potential resource competition between "Foxy-2" and indigenous microbes. The AFLP-marker revealed that soil type, organic resource availability and sampling date exhibited distinct effects on abundance of inoculated "Foxy-2". T. diversifolia residues increased "Foxy-2" and AMF abundance in both soils. We observed a stimulating effect of "Foxy-2" and *S. hermonthica* on abundance of archaeal nitrifiers, while bacterial nitrifiers remained unaffected. Furthermore, AMF species *Gigaspora margarita* abundance was stimulated by "Foxy-2" and supressed by *S. hermonthica* while other AMF species included in the study program remained unaffected. In conclusion, we confirmed the applicability of the AFLP-marker for monitoring the BCA Fos in two tropical soils and revealed that "Foxy-2" did not pose a suppressive effect on indigenous nitrifiers and AMF. To strengthen our findings obtained from this controlled rhizobox experiment, we recommend broad scale field studies to approve not only the robustness of the developed AFLP-marker, but also to assess the survival and actual side-effects of inoculated Fos in differently managed soils located in contrasting agro-ecological zones.

The microbial communities associated with potato rhizosphere under different seasonal conditions in South Africa

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Plant species have an ability to shape their rhizosphere microbial communities as determined by types of root exudates they release into the soil. In this regard, a PhD study at the University of Pretoria, South Africa, is currently investigating the bacterial and fungal microbial communities occurring in the potato rhizosphere as the season progresses. Two potato field trials were set up in a split plot in a Randomised Complete Block Design (RCBD) at the University of Pretoria experimental farm in the winter and summer seasons of 2014. Potato plants were cultivated as per regular agricultural practices. Potato rhizosphere soil samples were collected pre-planting, at tuber initiation, flowering and senescence stages. The samples were subjected to 16S and ITS Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis to determine the relationships amongst microbial communities from all plant growth stages compared to the bulk soil controls. The preliminary results from multidimensional scaling of TRFs obtained showed distinct patterns in microbial communities at different potato growth stages. This was observed for both bacterial and fungal communities in the two growing seasons. The plant senescence microbial DNA fingerprints were well separated from those of tuber initiation and flowering stages and were similar to the pre-plant samples. The pre-plant communities clustered together with bulk soil control samples portraying high similarity. This trend shows that the plants had an effect on the type of microbes surrounding their root system in their life cycle. However, this rhizosphere effect had a tendency to diminish towards plant senescence when the microbial structure reverted to its original state before introduction of the root exudates. The microbial communities associated with the root surfaces may play an important role as plant growth promoters, pathogens or biological control agents of soil borne diseases infecting those plants.

Soil microbes and their importance in shaping our forests using qPCR

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From nurseries to forests, soil microbes wield significant influence over the health and productivity of trees. By employing a comprehensive approach that integrates soil nutritional status and microbial community activity, it is possible to generate a clear depiction of the relationships between measurements of tree health and productivity and the endemic microbes present at a given site. This framework also provides an opportunity to assess the short and long term impacts of forestry management practices on soil microbial community properties, and the potential implications of any changes to microbial properties for forest growth.

Two long term experimental sites, located on a sandy and a gravelly soil, were established with a variety of replicated forest treatments plots, including variations in fertiliser use. For each treatment plot a bulked mineral soil sample was collected from 0-10 cm depth and sieved to attain the <2 mm fraction. The sieved soil was analysed for nutrients and soil microbial activity using MicroRespTM. To broaden the examination of microbial properties, DNA was extracted from the soils and quantitative PCR used to measure the gene copy numbers of bacteria and fungi, as well as the capability for nitrogen fixation and ACC deaminase production.

At the sandy soil site the range of carbon substrates utilised by soil microbes was significantly reduced with fertiliser addition compared to the no-fertiliser treatment; in the gravelly soil, this effect was absent. Quantitative PCR analysis of the microbial community identified large differences between the sites, most likely due to differences in soil type and climate. At both sites the fertiliser treatment had no effect on the bacteria and fungi gene copy numbers, with bacteria dominating fungi in all cases. However, copy numbers of ACC deaminase genes were increased by fertiliser addition at both sites, whereas the copy numbers of nitrogen fixation genes were decreased with fertiliser use.

This research has identified several long term responses of the soil microbial community to the addition of fertiliser. Further research using similar methodology is required to develop this concept further, thereby improving our ability to predict how the intensification of forest management will interact with the stability of the soil microbial functions that underpin forest productivity in both the short and long term.

Responses in Active Microbial Communities and Expression of Important Functional Genes in Forest and Agricultural Field Soil after Wood Ash Addition Revealed by Metatranscriptomics

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Wood ash is increasingly being produced as a by-product in different renewable energy processes and is regarded as a waste product due to its content of toxic compounds such as heavy metals (e.g. Cd, Pb, Ni). Wood ash does however contain valuable nutrients and possess soil-liming capabilities and wood ash is therefore utilized as a soil amendment. Increased use of wood ash as a soil amendment would lead to the return of more nutrients to the ecosystem where the ash originated from, making the whole process more renewable. Wood ash can however alter microbial communities and functions in soil systems and can thereby impacts on essential microbial driven processes involved in e.g. C and N turnover. This could lead to changes in the overall soil quality, but knowledge in this scientific field remains very sparse.
We have set up a microcosm experiment using both agricultural field soil and spruce forest plantation soil incubated at 10 °C for 100 days with different concentration of wood ash added (0, 3, 12 and 12 t ash . ha^{-1}). During incubation, soil has been collected for DNA and RNA extraction along with measurements of important soil parameters (e.g. pH, TOC, NO^{3-}, NH^{4+}).
Using a molecular metatranscriptomic approach very detailed insight into changes in the active microbial community (rRNA) from all three divisions of life and the expression of genes (mRNA) is achieved. The method is based on high throughput sequencing of total RNA extracted from soil followed by bioinformatic work of the sequences including quality filtering, assembly and annotation of contigs to databases in order to see where the sequences originates from. These results will, together with the results about changes in different important soil parameters, lead to novel and very detailed knowledge on how active microbial communities in the two soil systems responds to the addition of wood ash and will give knowledge on changes in the expression of functionally important genes in the soils.
This poster will present preliminary results and give an overview of current and future work to be done on this study.

Complex effects of altitude and exposure on microbial communities in (sub)alpine soils

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Mountain ecosystems are characterised by a high climatic variability, thereby affecting the principal biogeochemical soil processes with further implications on ecosystem functioning and soil microbial diversity. Indeed, the composition and diversity of soil microbial communities is expected to be greatly influenced by environmental factors such as altitude and exposure, as well as by the physico-chemical soil properties (e.g. parent material, pH) and vegetation cover. However, the influence of the above-mentioned factors on the soil microbiota in an alpine setting is still poorly understood.
The aim of this study was to investigate the effects of altitude (from 1200 up to 2400 m a.s.l.) and slope exposure (north and south facing-slopes) on the composition and diversity of soil microbial communities by using a climosequence approach. Furthermore, the soil properties that contribute the most to the shaping of the microbial community structure were determined. Ten subalpine-alpine sites, with 5 positioned at north and other 5 at south-facing slopes, were sampled in August 2012 in Val di Rabbi (Trentino, Italy). Along the studied elevational gradient, the vegetation varied from old-growth forests at lower elevations to alpine grasslands at higher elevations. All the sites were on acidic paragneiss parent material. A total of 450 samples were collected from the ten study sites. Three subplots (5x5 m) at 50 m elevational distance from each other were set-up at all 10 sites and 5 soil sub-samples were randomly taken in each subplot at 5 cm depth intervals (0-5, 5-10, 10-15 cm).
Soil microbial community structure in terms of diversity, evenness and richness was assessed by DGGE genetic fingerprinting for the three domains (Bacteria, Archaea, Fungi); while microbial abundance was quantitatively assessed by real-time PCR. We performed a multiple assay including eight hydrolytic enzymes that are involved in the C, N, P and S cycles. The principal physico-chemical soil characteristics were also determined. A multivariate statistical approach (nonparametric multidimensional scaling) was assessed to shed light into the relationships between soil microbiota and abiotic/biotic soil properties. Overall, our findings showed that bacterial abundances were more pronounced at south-facing sites, whilst Archaea were more abundant at north-facing sites. Fungal communities were influenced by the elevational gradient with a lower abundance in alpine than in subalpine soils.

Landscape proteogenomics: explaining the functional-phylogenetic relationships of microbial communities by gradients of organic C availability in soil

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The inputs of C and N from aboveground are considered limiting factors for the development of soil microbial communities. We hypothesize that the availability of energy sources in soil would influence the composition of microbial communities, as well as the cellular, metabolic and physiological capabilities of populations.
In this study, the relationships between the amount of energy sources (estimated as water-soluble C, WSC) and microbial biomass, community composition and physiological capabilities were studied in soil gradients located in South-East Spain (semiarid climate) including 20 soil samples (n=3, total of 60 samples). Soil samples include bare, shrubland and forest sites that differed consistently on organic C and N contents. A multidisciplinary approach including genomics, metaproteomics and phospholipid fatty acid analysis was carried out.
The bacterial biomass-dependence on WSC was not linear and saturated above 400 ppm of organic C. 16S RNAr gene analysis revealed that the composition of bacterial communities was shaped by the content of water-soluble C. For instance, Rhizobiales and Solirubrobacteriales increased in parallel to WSC. A similiar pattern was observed for Acidobacteriales, Corynebacteriales, Micromonosporales, Propionibacterales and Pseudonocardiales. In contrast, the relative abundance of Rubrobacterales, Cytophagales, Bacillales, Clostridiales, Chromatiales, Gemmatimonadetes, Oscillatoriales and Sphingobacteriales followed an opposite behaviour.
Canonical correlation analysis between the phylogenetic composition and the functionalities obtained by metaproteomics revealed a change in the structure of the soil microbial community that was linked to the gradient of water-soluble carbon and the abundance of non-dominant phyla (i.e Chloroflexi, Deinococcus or Bacteroidetes). Secondary metabolism and lipid transport and metabolism were among the most important cellular functionalities contributing to the differences between communities in constraining WSC sites. Further analyses will elucidate if the functionalities of each specific taxon are modulated (or not) by the amount of energy sources in soil.

Tree species effect on soil microbial community

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Understanding how tree species influence soil organic matter (SOM) dynamics is important to predicting how soil C sequestration and fertility will respond to management decisions and as well as global environmental changes that alter plant species composition across the landscape. Soil microbial community plays a major role in SOM dynamics, and different tree species may give rise to distinct microbial communities through differences in the chemistry of their litter, their mycorrhizal fungal associates and the exudates they release into the rhizosphere. We studied the influence of tree species composition in soil microbial community in a common garden of replicated monocultures of 3 broadleaf (*Quercus robur, Quercus suber, Betula pendula*) and 3 needleleaf species (*Pinus sylvestris, Pinus pinaster* and *Pinus nigra*) in north of Spain. Soil samples were collected at 2 different distances from single trees (10 cm as a rhizospheric soil and 1,5 m as a bulk soil) and at 2 different depths in the soil profile (0-5 cm and 5-15 cm). Abundance of microorganism was estimated via microbial biomass measurements, using the fumigation-extraction method for bacteria and ergosterol for fungi. The structural diversity of soil microbial community was analyzed using DGGE analysis of PCR- amplified 16S rDNA and fluorescence enzyme assays to determine the functional diveristy of microbial community. The results will be presented for discussion in the congress.

Carbon substrate utilization and microbial biomass in European forest soils are related to tree species diversity

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Tree species influence biogeochemical cycling through element deposition (throughfall, litterfall), root decomposition and exudates, and through their influence on the microbial activities in the soil. Yet, the effect of mixing tree species on soil functioning is unclear, in particular concerning the microbial diversity and activity in soils.
Here we synthesize results from the Exploratory Platform of the FunDivEUROPE project (http://www.fundiveurope.eu/). This network of 209 comparative plots covering tree diversity levels of 1 to 5 species was established in existing mature forests in 6 European regions. These six focal regions represent a gradient of major European forest types from boreal to Mediterranean forests. The aims of this study were to determine the soil microbial biomass and metabolic diversity of soil bacteria for these 6 European forest regions, presenting each a tree species richness gradient and to analyse the impact of tree species richness and the role of other controlling factors.
We analysed the relation between tree species diversity, the proportion of coniferous tree species and soil factors (pH, soil organic carbon, water soluble carbon and nitrogen) and the carbon substrate utilisation pattern of soil bacteria (BIOLOG Ecoplate), soil microbial biomass (fumigation-extraction), hot water carbon and nitrogen in the forest floor and the upper mineral soil horizon (linear mixed models, GLM for multivariate abundance data, discriminant correspondence analysis).
Mean values of microbial biomass carbon ranged from 3264 (Italy) to 8717 (Finland) mg kg^{-1} in the forest floor and from 465 (Italy) to 3748 (Finland) mg kg^{-1} in the mineral soil. Statistical models predicted microbial biomass to increase in both soil layers by 7-8% with each step increase in tree diversity. Increased proportion of conifers was linked to a decrease in the number of carbon substrates used by soil bacteria. The types of carbon sources used were dependent on region, proportion of conifers, soil pH and water-soluble carbon and nitrogen.

Drying treatment of soil samples affects DNA recovery but does not change the fungal community structure by metagenomic analysis

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High-throughput sequencing techniques have recently allowed fungal ecologists to answer many essential questions about the ecology and biology of fungal communities in forest soils. Several efforts have been made to standardize fungal metabarcoding studies, from the sampling to the final ecological interpretation. However, there are basic methodological questions that have not been solved yet, such as the sample handling or soil processing. In this paper we designed a species-specific hydrolysis probe to detect and quantify the extraradical mycelium of the edible ectomycorrhizal fungus Lactarius vinosus in soil samples using Real-Time PCR, but also to study how the most common soil dry treatments (Freeze-drying at -20°C, drying at 60°C and drying at 20-25°C) affects the DNA recovery of this species. We also massively sequenced the fungal community using the PacBio® RS II system based on the Real-Time (SMRT®) technology to evaluate the possible effects derived from the three dry treatments applied to each soil sample. Our results indicate that soil dry treatments affected DNA yield from *L. vinosus*, which was the highest for the freeze-dried samples, whereas drying at 60°C and 20-25°C showed lower values. However, Multivariate analysis did not show any dry treatment effect to the fungal community composition, with only 3% of the observed variation explained by the treatments. The analysis revealed that the samples from the same plot were clustered together, independently of the dry treatment. In addition, dry treatment did not affect richness nor diversity indices. These results suggest that soil dry treatment may affect DNA extraction efficiency or may cause a general DNA degradation to the entire fungal DNA, but a fast drop in the humidity content during all the soil dry treatments would not allow saprotrophs to feed on ectomycorrhizal mycelia. This study highlights the importance of choosing the appropriate soil dry treatment depending on the purpose of the research. The results obtained also validate the use of Real-Time PCR for specific DNA quantification of *L. vinosus* and the high-throughput sequencing platform Pac Bio® RS II, which could potentially overpass the sequence length biases associated with other sequencing platforms.

Using fungal and bacterial growth to evaluate the effects of ash application on forest soils

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Wood ash has been proposed as a liming material and a fertilizer suitable for acidic forest soils. However, the use of wood ash in forestry has been questioned because of the potential risk associated with heavy metals content. Ash application to soil results in several parallel effects, including increases in soil pH and elevating metal concentrations. This makes it a challenge to link effects to causal mechanisms. Soil pH is one of the most influential factors that structure microbial communities in soil, and it is also well-known that heavy metals strongly affect soil microbial communities, highlighting potential effects of ash application on microbial communities and associated functions. The aim of this study was to evaluate the effects of wood ash on soil microbial communities by: assessing the responses of bacterial and fungal growth in soils where different concentrations of wood ash were added; and by characterizing how ash application has modulated the pH dependence and cadmium (Cd) tolerance of soil microbial communities. In parallel, we also investigated these effects in laboratory microcosm studies. In these, we compared the effect of wood ash, and contrasted them with the effects of Cd and lime in a factorial design. These experiments were thus designed to disentangle the pH and toxicological effects of Cd in ash amendments. The results from in situ and microcosms studies were consistent and complementary. Bacterial growth increased and fungal growth decreased with higher rates of ash application. The bacterial stimulation was probably caused by a direct pH effect combined with an increase in carbon availability, as suggested by stimulated respiration, while the fungal responses could be related to competitive interaction with the stimulated bacteria. The ash application also modulated the pH relationships of bacterial communities, consistently shifting them toward more alkaline values. These results suggested that a substantial fraction of the microbial community response was a direct pH-effect. In the microcosms we found a similar shift in bacterial pH relationships in the ash and lime treatments. We were able to induce tolerance to Cd in the microcosm systems exposed to both lime and Cd, but found no evidence suggesting that the ash treatments affected the microbial tolerance to Cd. With these results we conclude that ash changes microbial communities due to the increase of pH but has no toxicological effects related with tolerance to Cd.

Winter in a changing climate affecting the survival of Scots pine seedlings

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Climate change scenarios predict increasing precipitation and air temperatures at high latitudes, particularly during autumn, winter and spring. The trends for soil temperatures, however, are more difficult to predict, as they strongly depend on the fate of the insulating snow cover, in regard of timing and depth. Warm periods during winter can result in thaw-freeze cycles and flooding, and eventually in the formation of ice layers, affecting e.g. concentrations of soil gases and microbial activity and also the survival of tree seedlings. We conducted a laboratory experiment, simulating winter, spring and early summer, and imposed 1.5 year-old Scots pine seedling to four different winter scenarios: (1) ambient snow cover, (2) compressed snow and ice encasement, (3) flood and (4) no snow. The soil was frozen during winter in all treatments. In addition to determining the biomass of needles, stems and roots, as well as their nutrient and carbohydrate content, we also measured the stress that the seedlings experienced by means of gas exchange, chlorophyll fluorescence and electrical impedance spectra of the needles. We also determined nutrient concentrations in the soil. The seedlings growing under the snow or compressed snow survived until the end of the experiment, although only those under the ambient snow cover showed normal height growth. The seedlings in the other treatments had died at the end of the experiment. Our results suggest the crucial importance of the protective snow cover, and that soil hypoxia and anoxia during winter as well as respiratory losses and winter desiccation of aboveground organs were the most probable reasons for the death of the seedlings.

Pyrosequencing based assessment of bacterial and fungal community compositions in compacted and regenerated forest soils

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Soil compaction has been recognized as a major disturbance associated with timber harvesting and we currently lack fundamental knowledge how these perturbations affect soil microbial diversity. In order to predict the ecological consequences of soil compaction as well as to evaluate the capacity of soils to recover naturally or after planting compacted soils with Alnus tree seedlings, we assessed the structural resistance and resilience of the soil microbiota using a high-throughput pyrosequencing approach. Compaction significantly reduced abundance, increased diversity, and persistently altered the structure of the microbiota. Fungi were less resistant and resilient than bacteria; clayey soils were less resistant and resilient than sandy soils. The strongest effects were observed around 6 to 12 months in the severely compacted wheel tracks where air and water conductivities were reduced permanently to 10% or even lower of the original conductivities of undisturbed soils. Seven years post-disturbance showed resilience in lightly but not in severely compacted soils. Bacteria capable of anaerobic respiration, including sulfate, sulfur, and metal reducers of the Proteobacteria and Firmicutes, were significantly associated with compacted soils. Compaction detrimentally affected ectomycorrhizal species, whereas saprobic, parasitic, and mutualistic fungi proportionally increased in compacted soils. Structural shifts in the microbiota were accompanied by significant changes in soil processes, resulting in reduced carbon dioxide, and increased methane and nitrous oxide emissions from compacted soils. Monitoring response and resilience of key microbial groups over time and after restoration measures, helps to predict the extent and persistence of soil damage and facilitate the development of efficient restoration strategies of compacted soils.

Soil ß-Glucosidase activity under canopy of White Poplar in riparian forests

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Soil microorganisms produce and release extracellular enzymes that play important role in organic matter degradation and nutrient cycling. The enzyme activity may be influenced, directly or indirectly, by environment conditions and can be used as early, sensitive indicators of changes in soil ecosystem functioning. ß-Glucosidase (EC 3.2.1.21) plays an important role in the soil functioning as it is involved in the last step of cellulose degradation. The released products provide a source of carbon for soil microorganism. White Poplar (*Populus alba* L.) is one of the main tree species forming riparian forests in river valleys. Construction of flood embankments, dams, river regulation caused a reduction or complete inhibition of flooding which resulted to the disappearance this unique ecosystem, heavily depended on the annual flooding of rivers. The aim of this study was to determine activity of ß-Glucosidase on natural riparian stands, characterized by repeated flooding and the sites where the river flooding were stopped by human activities. Samples were collected in spring and autumn 2011 (one year after the great flood) from three soil depth: 0-10, 10-20 and 20-30 cm. In the spring season, enzyme activity was significant higher in the soil from regularly flooded site than from the location without flooding. The highest ß-Glucosidase activity was observed in the upper level of the soil profile at each of the study sites. Activity of ß-Glucosidase was significantly positively correlated with soil moisture.

Spatial variation of the fungal metagenome in temperate beech forests across Germany

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Fungi play an important role in the functioning of terrestric ecosystems. They may serve as pathogens or more likely as symbionts and saprobes. Changes in the soil fungal community composition has been shown to be influenced by plant community composition at different spatial scales. However, little is understood about other environmental variables affecting the biogeographical patterns of belowground fungi in forests dominated by the same tree species. To fill this gap of knowledge, we are currently studying fungal communities in three temperate regions. Our study covers beech dominated forest experimental plots within three large-scale and long-term research sites across Germany. Those sites were established by a large interdisciplinary consortium funded by the German Science Foundation – DFG called the German Biodiversity Exploratories. Furthermore a broad range of management types varying from unmanaged natural forests to highly managed timberlands complete the design. We assessed the fungal community structures and diversity in each of the forest plots using high-throughput amplicon pyrosequencing, targeting the ITS region of the fungal rDNA as marker.
First results showed that the fungal communities are significantly affected by various biotic and abiotic factors. Furthermore, we expect spatial changes in the fungal community composition across sites and regions.

Soil microbial community changes in the disturbed Norway spruce stands during a 10-years period

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In autumn 2004, Norway spruce stands in Tatra Mts. (Slovakia) were completely destroyed by wind on the area of 12.000 ha. Several months later, a part of this area was burnt by wildfire. Shortly after the disturbance, long-term research plots were established on the affected area with the aim to perform interdisciplinary and international research regarding the moitoring and study of the changes in all land components including soil microbiota after the disturbance. Study was performed at four research plots: 1/plot with removed fallen trees, 2/plot with removed fallen trees and burnt by fire, 3/plot left without management, without removing fallen trees, 4/reference plot, forest stand not affected by wind. Plots are situated at the elevation of 1,000–1,250 m a.s.l. Soil samples were taken at 10 m intervals along 90 m long transects at each plot from the mineral A- horizon 1 to 3 times during each vegetation period since 2006. For the characterisation of soil microbial community, microbial biomass, basal respiration, SIR, N-mineralisation, catalase activity and richness and diversity of microbial functional groups based on Biolog assay were determined.
We tested the temporal (inter-annual) trends of microbial characteristic employing linear regression to find out whether there is any increase or decrease of microbial activity at study plots during the observed period. SIR, microbial biomass and N-mineralisation showed a significant linear trend with a slight increase observed at all windthrow plots what indicates slight recovery of the microbiota after windthrow. Although there are several significant differences in characteristics of microbial activity between plots, their pattern is not consistent during the observation period, what can be explained by gradual succession of plant communities at the disturbed plots and, consequently, changes in the organic matter input and microclimate. No significant differences among plots were found in the richness and diversity of functional groups. Abundances of some microbial groups as measured by specific substrate utilisation decreased (α-cyclodextrin) or in opposite, increased (D-malic acid, D-mannitol, pyruvic acid methyl ester, phenylalanine, L-asparagine, L-arginine, N-acetyl-D-glucosamine, D,L-α-glycerolphosphate) from year to year. While increased soil acidity was reflected in decreased utilisation of α-cyclodextrin and D-cellobiose, the utilisation of itaconic acid and L-serine increased with pH.

CH_4 and N_2O microbial communities respond to site preparation and fertilization in wet forests

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Site preparation (mounding or drainage) and fertilization of wet forests can improve aboveground productivity, but this may be at the cost of increased soil-to-surface greenhouse gas (i.e. CH_4 and N_2O) fluxes due to changes in soil moisture, soil C and N concentrations and the soil microbial community. There are conflicting results regarding CH_4 and N_2O emissions following site preparation and fertilization, which could be resolved through study of the microbial functional groups responsible for regulating CH_4 and N_2O emissions, i.e., methanogens, methanotrophs, nitrifiers and denitrifiers. We assessed the effect of excavator mounding and fertilization of interior hybrid spruce stands, and drainage and fertilization of coastal western hemlock/western redcedar/yellow cypress stands on CH_4 and N_2O fluxes and the abundance of methanogen *mcrA* genes, methanotroph *pmoA* genes, ammonia-oxidizing archaea (AOA), bacteria (AOB) *amoA* genes and denitrification genes (*narG, nirK, nirS* and *nosZ*). Laboratory potential denitrification rates (PDR) were studied to determine the effect of site preparation and fertilization on enzyme activity, gene abundance and transcript abundance. Excavator mounding of wet forests reduced the abundance of denitrification genes (*narG, nirK, nirS* and *nosZ*) in soil while increasing AOA and AOB *amoA* gene abundance, while ditch drainage reduced AOA and denitrifier gene abundance. CH4 flux and mcrA abundance was also decreased by drainage. Nitrogen (N) fertilization, however, increased AOB and denitrifier gene abundance as well as N_2O flux rates. The increase in N_2O fluxes was linked to AOB abundance and denitrifier gene abundance and expression using potential denitrification incubations. CH_4 flux was also increased by fertilization and mounding. Between 63-90% of nitrification and denitrification gene abundance variation could be explained by soil physico-chemical and spatial-temporal variables following redundancy analysis and canonical variation partitioning. Functional gene quantification provides an improved understanding of soil process rates (e.g., CH_4 and N_2O flux) following management of forests.

Recovery of ectomycorrhizal community of a boreal forest after three decades of N fertilisation

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Nitrogen (N) is generally accepted to limit primary production in boreal forests. Increased N input (e.g. by fertilisation) changes trees C allocation and soil N availability, which commonly results in a decreased abundance of ectomycorrhizal fungi (EMF) and shift in the EMF species composition. As different EMF taxa have different life strategies, N loading can alter the functioning of the EMF community. With decreased N deposition in Europe during the last decades, recovery of EMF and shifts in EMF community have been considered. However, knowledge of process is currently very limited.
Our goal was to describe effects of N fertilisation and recovery after the termination of N loading on the EMF in an N-poor Norway spruce (*Picea abies*) forest site located in central Sweden (E26A Strosan). Three levels of N treatment were applied there in a randomized block design since 1967 – N0 (no N added), N1 (total dose 1710 kg $N.ha^{-1}$, ongoing) and N2 (total dose 1760 kg $N.ha^{-1}$, terminated in 1990). The mor layer from control and N treated plots was sampled in May and October 2013.
Contrary to our expectations, N fertilisation of strongly N-limited soil evoked only minor changes in the chemistry of mor layer: a decrease in the C:N ratio and an increase of NH4+ in comparison to control N0, with N1 treatment being more enriched in N than N2.
The sequencing of fungal ITS region showed significant decrease of EMF within the total fungal community on N1, but recovering tendency on N2. The EMF community on N0 was dominated by genus *Piloderma* followed by *Russula* and *Lactarius*. On N1, proportion of *Piloderma* and *Russula* was lower in favour of *Lactarius* and *Tomentella*. On N2, *Piloderma* was dominant again and also *Cortinarius*, present on N0 but practically absent on N1, expanded. Considering the ecology of particular fungal genera, N loading resulted in an expansion of EMF types able to quickly use available N in close environment over the types spreading their mycelia to longer distances, in search of limited sources, which dominated N0 plots. Termination of N loading enabled a restoration of longer distance types of EMF on N2.
Our results confirm generally accepted negative response of EMF proportion in fungal community to N additions and showed a potential of their recovery after the termination of high N inputs into the system. Still, the EMF proportion within the total fungal community is lower on N2 than on N0 and the genera composition differs after 23 years of recovery.

A new promising molecular marker to study the functional diversity of fungal communities: the GLYCOSYL HYDROLASE 63 gene

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Fungal communities are a key component of forest ecosystems, involved in biogeochemical cycling and tree productivity. Because resilience of forest ecosystems in response to environmental changes is dependent on ecosystem services provided by fungi, one of the goals of the community ecology is to predict the effects of disturbance (environmental change) on ecosystem function. Our main aim was to develop a new functional diagnostic molecular marker to identify fungi involved in carbon cycling and sequestration. Thus, we developed a series of primers to amplify the single-copy GLYCOSYL HYDROLASE GH63 gene, encoding exo-acting β-glucosidases, in Basidiomycetes. These primers were validated on 125 different fungal genomic DNAs and GH63 amplification yield was compared with those of already published markers targeting genes coding for laccases, N-acetylhexosaminidases (GH18), cellobiohydrolases (GH7), or class II Peroxidases (AA2). GH63 amplification success was strikingly higher than rates obtained with other functional genes. Specific amplicons were recovered for 95 % of the fungal species tested. We then downloaded the GH63 sequences from the 483 fungal genomes publicly available at the JGI MycoCosm database. GH63 was present in 461 fungal genomes (as single-copy gene in 86 % of them) belonging to all phyla, except Microsporidia. When comparing the phylogenetic trees built with GH63, Rpb1 or Rpb2 protein sequences, GH63 emerged also as a promising barcoding and phylogenetic marker. Finally, a high proportion of GH63 proteins was predicted to be secreted, especially within the phylum Basidiomycota. The added value of this molecular tool is that it will serve as new indicator of structure and functional diversity of fungal communities, enabling to provide new data about the role of fungi in the carbon cycle, as well as their potential secretory capacities.

Methane flux in wood ant (*Formica polyctena*) nests and the surrounding forest floor

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Methane (CH_4) is one of the most important greenhouse gases. Wood ants might be able to influence CH_4 flux in their nests thanks to high porosity, low moisture and stable temperature suitable for CH_4 oxidation. In our study, we sampled profiles (0-30 cm) of wood ant nests and of surrounding forest floor, incubated the substrates in the laboratory and measured the rate of methane flux. Additional substrate characteristics such as moisture, ammonia (NH^{4+}), nitrate (NO^{3-}) and water-soluble carbon (WSC) concentrations or abundance of methanotrophic bacteria via catalyzed reporter deposition - fluorescence in situ hybridization (CARD-FISH) were also determined.
Contrary to our expectations, CH_4 production prevailed in substrates from wood ant nests. In the surrounding forest floor, CH_4 oxidation prevailed as expected. CH_4 production and CH_4oxidation increased with the depth in wood ant nests and forest floor, respectively. NH^{4+}, NO^{3-}, and WSC concentrations were positively correlated with CH_4 flux encouraging CH_4 production. Only type II methanotrophic bacteria were found in the substrates, no type I methanotrophic bacteria. Abundance of these bacteria, however, did not explain CH_4 flux in the substrates. There are probably another types of methanotrophic bacteria responsible for CH_4 oxidation in forest floor substrates.

Metabolite profiles of soil actinobacteria follow their phylogeny and environmental factors at the isolation sites

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The study was conducted to show that cultivable strains of actinobacteria differ in phylogeny, metabolite profiles and antibiotic activity, when isolated from soils spanning over a range of pH and organic matter contents.
A set 336 isolated strains of actinobacteria were isolated at ten soil sites, phylogenetically characterized by sequencing the genes for 16S rRNA (*rrs*) and the beta-subunit of DNA-dependent RNA polymerase (*rpoB*). The strains were cultivated in submerged culture and the spent media were used to determine the inhibitory activities against *Kocuria rhizophilla*, *Escherichia coli*, and *Saccharomyces cerevisiae*, directly or after solid-phase extraction. In the solid-phase extracted fractions, the profiles of low-molecular weight metabolites were determined by reversed-phase HPLC with UV-VIS diode array detection.
The strains differed in their phylogeny by the site of isolation ($P<0.001$). Their HPLC profiles were related to the phylogeny, both for all actinobacteria and for a subset of streptomycetes, and to the site of isolation (for all, $P<0.001$). An effect of the isolation site was observed also for the antibiotic activities against *S. cerevisiae* ($P<0.05$), with the strains from an acidic pine forest soil being the most active. Further, the antibiotic activity was related to the *rpoB*-based phylogeny for all testing strains ($P<0.001$ - 0.01). For the *rrs*-based phylogeny the observed relationships were weaker, while only the relationship to the activity against *K. rhizophilla* remained highly significant ($P<0.001$).
The results demonstrated a strong relationship of the strains antibiotic activities and profiles of low-molecular weight metabolites to their phylogeny, both for all actinobacteria isolates and a subset of the genus *Streptomyces*. They showed that phylogenetic differentiation was the main mechanism determining the strain properties.

Relations between *Peltigera* lichen's derived factors and its associated bacterial communities

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Diversity of the symbiotic members of lichens has been extensively studied and described; however, little is known about the diversity of microorganisms inhabiting the lichen-provided habitats. It has been proposed that lichen-associated bacterial assemblages could form two differentiated microbial components: (i) the intimately-associated lichen microbiome, which corresponds to bacteria living tightly-associated with the lichen thalli, and (ii) the lichen-adjacent soil microbiota, which corresponds to those microorganisms residing in the substrate that supports the lichen (e.g. soil).
In this work it is proposed that the intimately-associated lichen microbiome structure would be under the influence of lichen (e.g. metabolite and enzyme activities), contrasting with the structure of the lichen-adjacent soil microbiota, which is rather expected to depend on environmental conditions defining the substrate properties (e.g. edaphic conditions).
In order to test this hypothesis, first, 50 *Peltigera* lichen thalli and their corresponding associated substrates were randomly sampled from two sites of a *Nothofagus pumilio* forest in the Coyhaique National Reserve, Southern Chile. Using ribosomal gene markers, 6 mycobiont and 5 cyanobiont haplotypes were found, and consequently 11 composite samples were defined.
Then, on each lichen composite sample, lichen metabolite diversity was analyzed by TLC-2D and phenoloxidase activity was measured by spectrophotometric analyses. Finally, genetic and metabolic structures of the bacterial communities related to lichens and substrates were determined by TRFLP and CLPP, respectively. Genetic and metabolic structures were different, depending on the symbiont identities and the forest from where they derived. In addition, correlations were found between diversity of lichen metabolites and genetic structures of bacterial communities associated to both thalli and substrates, and between lichen phenoloxidase activities and the metabolic structure of bacterial communities associated to thalli.
Results of this research suggest that metabolite production and phenoloxidase activity, both factors attributable to lichen, would have a role in determining the genetic and metabolic diversity of the bacterial communities associated to *Peltigera* lichen thalli and to the substrates where they grow.
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Spatial variability of soil microbial processes in a temperate mixed forest

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Spatial heterogeneity in forest soils results from differences in quantity and quality of substrate input through litter and root exudates, the influence of trees on soil texture/structure, variable microclimatic conditions under canopies and from the development of a specific understory vegetation. This spatial heterogeneity of environmental conditions may affect microbially mediated processes such as nutrient cycling or organic matter decomposition. Although a number of studies report tree influence on soil microbial activities, information about spatial variability are still scarce. The variability in soil functioning limits exact quantification of the soil processes and hampers the establishment of a relationship between microbial diversity and function.

The main purpose of this study was to describe the spatial variability of soil microbial processes and their relationship with soil characteristics across a 1.5 ha plot of a temperate mixed forest in Belgium. We evaluated which soil properties were the most important in regulating microbial processes at different spatial scales. 99 samples from the forest floor (Oh) were taken at distance ranging from 0.5m to 60m within the study plot (120m*120m) using a nested sampling design strategy. The stand was covered with oak (*Quercus petraea* LIEBL) and beech (*Fagus sylvatica* L.). Samples were analysed for microbial (net N mineralization, potential nitrification, basal respiration, microbial biomass, microbial or metabolic quotient) and soil parameters (pH, extractable ammonium, organic matter content, cold water C and N and hot water C and N).

Here, we report the results of a hierarchical analysis of variance based on residual maximum likelihood (REML) in order to identify the scale important for the variation of microbial processes. This method allows the description of the general form of spatial dependence. Moreover, the scale-dependant covariation of microbial properties with soil parameters is addressed using cross-variograms.

Fungal communities associated with rhizosphere of *Nothofagus alpina* from different volcanic ash-derived soils in southern Chile

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Rhizosphere fungal communities play an important role in growth, health and survival of plants. However, there is scarce knowledge about fungal richness in volcanic ash-derived soil ecosystems, and the soil chemical parameters that regulate fungal community structure. In the present study, we analyzed the diversity of fungi inhabiting the rhizosphere of Nothofagus alpina forest located in Chilean ash-derived volcanic soils: Andisols (Pelchuquin, San José, Lumaya and Liquiñe soil series) and Ultisols (Los Ulmus soil series) in Los Rios region (Valdivia, Chile). *N. alpina* is one of the most important species of Patagonian temperate forests. Fungal community composition was determined using Illumina MiSeq system sequencing of the internal transcribed spacer 1 (ITS1). Soil chemical parameters were measured by standard methods. We observed significant differences ($p<0.05$) in the chemical parameters in the study soils through PCA and ANOVA. The diversity of fungal communities, using Shannon index and Pillou index, was not significantly different between the soil series regarding the operational taxonomic units (OTUs). PERMANOVA analysis showed significant differences between fungal community structure inhabiting different soil series. Among all chemical parameters, only the soil organic matter (SOM) content significantly influenced the fungal community structure. These results suggest that SOM content may be a more important factor than pH or soil nutrient status (N, P, K, etc.) that regulate fungal community structure in the rhizosphere of *N. alpina* located in volcanic ash-derived soils.
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Development and Decline of Microbial Communities Associated with Ectomycorrhizal Mats

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Certain ectomycorrhizal fungi (EmF) form specialized profusions of hyphae, known as ectomycorrhizal fungal mats (EFM) that are visible to the naked eye, alter forest soil biogeochemistry, substantially contribute to soil microbial biomass/respiration and support unique microbial communities. Piloderma EFM stratify in organic horizon and are the dominant mat-forming fungi of old-growth Douglas-fir forests of the Pacific Northwest, USA. The importance of Piloderma EFM to forest ecosystem processes has driven the need to better understand EFM associated microbial communities, particularly their development (birth) and decline (death) dynamics. To explore these dynamics, a reciprocal soil transplant experiment was established at seven old-growth Douglas-fir sites in the H.J. Andrews Experimental Forest. At each site Piloderma and non-mat soils underwent birth (non-mat into mat enclosed in 2 mm mesh), death (mat into non-mat enclosed in PVC pipe), disturbance control (core non-mat soil, replace in 2 mm mesh) and background (no manipulation) treatments. After 51 months, treatments were harvested and three microbial community segments were assessed through molecular analyses: (1) active EmF root-tips, Sanger sequencing; (2) soil fungi and bacteria, 454-pyrosequencing. Piloderma mat fungal communities persisted for 51 months and remained distinct from non-mat soils; this permitted birth and death treatment analysis. Our data indicate strong development of Piloderma mat fungal communities in birth treatments, beyond colonization by Piloderma, making them indistinguishable from Piloderma mats; mat development can take many years. Death treatments were dissimilar to Piloderma mats and contained similar fungal communities to non-mat soils. Enclosure in PVC pipe, thereby removing roots and EmF from the system, significantly shifted the soil fungal community toward saprotrophic dominance. For organic horizon bacterial communities, only death treatments differed from others; strong similarities were found between overall Piloderma mat and non-mat bacterial communities. However, Piloderma mat and non-mat soils impose selection pressure on a small subset of bacterial taxa masked when the community is considered as a whole. This work contributes to the body of knowledge regarding complex microbial community dynamics of EFM. The occurrence and distinct taxa of Piloderma mats in these forests suggests large-scale spatial differences in microbial community ecological function.

The importance of fungal-fungal and bacterial-fungal interactions for phosphorus dynamics in forest soils

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Plant uptake of phosphorous (P) in complex forest ecosystems relies to a great extent on microbial mineralization of P from organic and inorganic sources. Multiple interactions within the soil community could thereby stimulate or reduce the P available for plant growth. In this connection the fungal-fungal and bacterial-fungal interactions play a decisive role.
We hypothesize that: (I) organically bound P substrates promote antagonistic effects between saprotrophic fungi (SP) and ectomycorrhizae (EM) due to strong P competition. (II) Inorganically derived P compounds are mobilized by bacteria; therefore synergistic effects between fungi and bacteria are induced. An accumulation of associative bacteria and fungi cause structural differences between the microbial community compositions.
To proof our hypothesis we performed a systematic exclusion of EM in the field at five beech-wood forest sites by the use of a meshtube trenching experiment. It is hypothesized that the selected sites (BBR, CON, VES, MIT and LUE) form a gradual shift in allocating P from recycling systems (BBR) to acquiring systems (LUE). We installed eight meshtubes in spring 2014 (four “open” and four “closed” tubes) into the upper soils in a distance of 2.5 m to five *Fagus sylvatica* L. trees, respectively. The tubes ensure selective fungal hyphae ingrowth. Open tubes are surrounded by a 50 µm mesh-window to permit access to EM-hyphae. Closed tubes prevent for lateral fungal ingrowth. Both tubes comprise the native fungal SP community and were exposed for a period of 15 months. After 3, 6, 12 and 15 months, five tubes of each treatment and site are removed together with five additional undisturbed soil samples.
Tube derived soils and control samples were analyzed for: (1) microbial bound P contents (Pmic), (2) potential phosphatase activities (PAs) and (3) microbial community composition (PLFAs). First results after 3 and 6 months showed that the sites differ in their potential to mineralize organic P compounds and in their microbial community composition. The Pmic fractions as well as the PAs showed slight differences between the sites. The fungal PLFA marker (18:2?6,9) followed the anticipated shift in P-allocation from BBR to LUE. Slightly higher fungal abundances in the open vs the closed variants indicating a successfully EM exclusion. A further treatment validation by molecular analyses (qPCR, pyrosequencing) is planned as the next step.

Fine scale modification of soil physical properties by fungi: reinforcement and repellency in the hyphosphere

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In forest soils, fungi represent a considerable proportion of the microbial biomass and modify soil physical properties, the combination of which could have major implications for the functioning of forest soils, such as nutrient cycling. The assessment of such modifications is usually conducted by mechanically destructive techniques such as sieving. This technique provides some insights into the scale that fungal effects are most prevalent, however, the location of where the stabilisation occurs in the sample in relation to the mycelium is unknown. Knowledge of mechanisms driving these observations is therefore limited. Yet, understanding these mechanisms is important in determining how forest systems will respond to perturbations.

Here we test how soil fungi modify the surrounding soil via mechanical reinforcement and hydrological processes, while maintaining spatial information. Applying theories and methods from materials science will generate quantitative data from which mechanisms such as exudation and enmeshment can be modelled. We also test how different species traits and ecological strategies relate to modification of soil physical properties. The study utilised a sandy forest soil, with a well-characterised fungal community, alongside a collection of species-specific microcosms containing either ectomycorrhizal or saprotrophic fungal species. For the field component, treatments were established using nylon mesh to provide root-free soil with or without extraradical mycelium. The in-growth cores were left for 6 or 9 months before retrieval.

Mechanical reinforcement of the soil was quantified using a 3 mm spherical indentor. The samples were progressively loaded by 10 load/unload cycles to a depth of 1 mm and the force recorded throughout. Soils containing fungi had an average resistance 2 to 6 times greater than soils without fungi. The distribution of this reinforcement was heterogeneous across the surface with some areas showing little difference from non-fungal treatments. A mini infiltrometer was used to make fine scale measurements of the surface hydrology of each sample. Water sorptivity did not differ between the field treatments. This could be masked by the soil already being hydrophobic. These techniques are currently being applied to species-specific microcosms. This study demonstrates that modification of soil by fungi close to the mycelium can be substantial and provides a base for mechanical understanding of this process.

Multi-dimensional mycelia interactions

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Wood decay occurs by a community of fungi which interact antagonistically with each other, causing the community composition to change. Since these fungal interactions are, therefore, a decisive force in aspects of forest ecology such as nutrient cycling, soil formation and carbon budget, understanding these interactions is crucial. In the past, interactions have usually been studied by pairing fungi in different interactions, but this is unrepresentative of the real world, where many fungi interact simultaneously. The presented study, therefore, simulates multiple fungal interactions in 3 dimensions, by constructing “Rubik’s cube” configured systems from 2x2x2 cm wood (*Fagus sylvatica*) blocks pre-colonised with fungus in a 3x3x3 cube. Species are spatially arranged with, for example, a primary resource colonising species forming an entire layer of the cube, and a secondary resource coloniser and a cord forming species dispersed throughout the remaining two layers. Rubik’s cubes are deconstructed to determine whether fungi have been replaced by others, CO_2 evolution and volatile organic compounds will be measured, and the expression profiles of key genes analysed to gain an insight into the outcomes of multiple mycelia interactions within this multi-dimensional system.

Dynamics of *Boletus edulis* extraradical soil mycelium and sporocarp production in managed forests

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Extraradical soil mycelium and sporocarp production of the king bolete (*Boletus edulis* Bull.) were regularly recorded in a *Pinus sylvestris* L. forest in Soria (central Spain) from 2008 to date. The obtained dataset allowed to establish correlations between vegetative fungal persistence (extraradical soil mycelium), sporocarp production, and climatic parameters (monthly mean precipitation and temperature), and to determine the effect of forest management practices (seasonal sporocarp collection and cutting for timber production) in *B. edulis* mycelium biomass. Extraradical soil mycelium was specifically quantified by real-time PCR in plots submitted to complete and partial clearcutting and in undisturbed areas. In a parallel experiment, intensive collection of *B. edulis* sporocarps (all the fruiting bodies were removed weekly) was done along the fruiting season of four years (2011-2014) and the effects on soil mycelium biomass were compared to those in fenced plots in which no collection of *B. edulis* sporocarps was done. Extraradical mycelium biomass was negatively and significantly correlated with the precipitation recorded two months before, possibly due to the temporary flooding which periodically occurs in the study area. No other significant correlations were found between the mycelium biomass and the temperature and precipitation recorded in the month of sampling or 1 to 6 months before. Sporocarp production was strongly and positively correlated with mycelium biomass and precipitation in autumn, but no significant effect of temperature in fruiting of *B. edulis* was observed.
Complete and partial clearcutting severely depleted the soil mycelium biomass of *B. edulis* as compared to control, undisturbed plots. Mycelium recovery was not detected after 24 months since timber removal. Intensive collection of B. edulis sporocarps along four productive seasons did not significantly affect the soil mycelium biomass as compared to non-collected plots. According to these results, an integrated forest management taking into account sustainability and multiproductive criteria is necessary to improve ecosystems services and soil conservation.

Virus host switches between pathogenic, mycorrhizal and saprotrophic fungal species in a boreal forest

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Viruses are common among fungi, but as most of them are cryptic, their ecology is poorly known. There exist only few reports on suspected viruses in mycorrhizal fungi, mostly observed due to an altered phenotype of the host fungus. In contrast to other organisms, transmission of viruses between fungi is considered to be very limited owing to vegetative incompatibility of mycelia between even conspecific fungal strains. Mycelia of coniferous pathogen complex *Heterobasidion* spp. are known to be long-lived and possess great variety of viruses. Therefore we studied the potential of fungal virus transfer between different host species at the forest site where spatial distribution of clonal individuals of *H. parviporum* and associated viruses was known to be high - the overall viral infection rate is 48-67% (Vainio et al. 2015. ISME J. 9:497–507). A total of 186 mycorrhizal and saprotrophic fungal fruiting body samples, 259 mycelial samples collected from mycorrhizal in-growth mesh bags incubated at the study site, and 68 fungal isolates were examined. The majority of the fruiting bodies represented mycorrhizal fungal species of *Lactarius, Russula* and *Paxillus*. The presence of Heterobasidion viruses was examined by RT-PCR and sequence analysis, and previously unknown viral dsRNA elements were identified by virus genome sequencing. 454-sequencing of the mycorrhizal mycelial in-growth-bags was performed to rule out the possibility of contaminating Heterobasidion mycelia. We found *Heterobasidion partitivirus* strains both from mycorrhizal mycelia and fruiting bodies of *Lactarius tabidus* and *Rhodocollybia butyracea*. Also for the first time, novel virus strains distantly related to known *Heterobasidion* viruses were found among *L. tabidus* and *L. rufus*. Interestingly, these viruses are somewhat related to the Curvularia Thermal Tolerance Virus, the only strictly mutualistic mycovirus known to date. As we have previously found mycelia of *Lactarius* sp. to be surprisingly abundant in decaying wood (Rajala et al 2011. Fung Ecol 4:437-444), potential for mycelial contact between these specific fungi is high, irrespective of the fact that they belong to different functional guilds. We suggest that the networks of fungal mycelia in the forest soil organic matter may not only serve as a potential for resource distribution among plants but may also function as a distribution route for viruses.

Arbuscular mycorrhizal fungi in protected areas of northeastern Brazil

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Arbuscular mycorrhizal fungi (AMF) constitute a group of symbiotic organisms essential for the maintenance of natural plant ecosystems and can be used as biological indicators of soil and habitat quality. The Atlantic Forest in Brazil is one of the most threatened habitats on the Earth, with less than 12 of their original areas still existing. Protected areas in this habitat are essential to maintain the biodiversity that remains. Thus, this study aimed to determine the AMF communities' structure in ten Protected Areas belonging to the Atlantic Forest (5 Strict Protection and 5 Sustainable Use) distributed in five states in Northeastern Brazil: Rio Grande do Norte, Paraíba, Pernambuco, Sergipe and Bahia. In 2013 and 2014 soil samples were taken in four plots of 5m x 20m. AMF spores were extracted from the soil, quantified and identified morphologically. Fifty eight species of AMF were found in both Protected Areas (40 in Strict Protection and 46 in Sustainable Use area). The sampling effort of the present study was sufficient to recover 62-69% of the AMF species present in the areas. The genus *Glomus* dominated in both areas representing almost 33% of the identified species. Despite a higher richness in the area of Sustainable Use the AMF community structure data showed a lower evenness and diversity due to dominance of some species. The impacts on the Sustainable Use area such as human pressure and the presence of invasive plant species, may have contributed to the change in species composition of AMF, which may in turn affect plant species responses to AMF. Ongoing work is using molecular analyzes of the AMF community present inside the roots. The AMF community composition could be used as a biological indicator of conservation status of the protected areas of the Brazilian Atlantic Forest with the data on occurrence and distribution of AMF species as a valuable parameter in the administration and management of these protected areas.

A first look at the *Quercus suber* (cork oak) root microbiome: differences between healthy and declined trees

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Oak forest decline is a major concern due to the social and economical importance of these trees in the Northern hemisphere. Triggered by a complex interaction of biotic and abiotic factors, tree decline needs to be addressed by new multifaceted approaches and perspectives. The role of tree-microbiome interactions has been highlighted as significant to the overall system stability and plays an essential role in tree homeostasis thus changing the mindset in forest ecology and management.
In this study, the cork oak root microbiome was analyzed by 16S and ITS amplicon pyrosequencing and a rich bacterial and fungal community was unveiled. Healthy and declined trees showed significant differences in the rhizosphere microbiome. Healthy cork oaks were dominated by ectomycorrhizal fungi while an increase of saprobes was observed in declined trees. In the bacterial community, particular functional groups, such as nitrogen-fixing, anaerobic ammonium-oxidizing, sulfate-reducing and plant-growth promotion bacteria, contributed significantly for differences between healthy and declined trees. These preliminary results support the hypothesis that tree health states have specific microbiomes and motivate further studies to clarify the role of the microbiome on cork oak health.
An ongoing project aims the tree rhizosphere microbiome study using the cork oak forest as a decline model. Throughout the seasons and in different geographic locations, bacterial and fungal communities will be analyzed by metagenomics approaches in healthy and declined cork oaks and correlated with temporal-spatial factors. Furthermore, root microbiome of different tree decline stages will be assessed and correlated with forest decline progression. Results derived from this project will provide new insights into the definition of a healthy root microbiome, unraveling potential microbes with an important role in tree growth, health and productivity. With this knowledge, new forest management strategies may be developed such as the use of probiotic microbial consortia for forest regeneration, disease suppressive soils to control phytopathogens or microbial markers for forest decline.

Overlap in the metabolic functions of cellulose-decomposing leaf litter fungi

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Key-stone fungal species could have their force in the fact that they behave as pioneer on particular substrates (like lignin, cellulose, collagen). Actually several studies showed that micro-habitats and substrates are of considerable significance in determining fungal species' distribution and for some authors local differences in distribution are greater than continental differences. This means that the scale at which fungal ecology concepts are tested against environmental sampling is determinant, and the comprehension of patchiness in fungal partitioning, competition and variability is fundamental.
A technique for trapping celluloytic fungi at the soil-leaf litter interface was set up. The system was incubated for 45 days under leaf litter originating from an area of a low mixed Mediterranean maquis located in southern Italy. Four different sites as field replicates were considered. The micro-fungi that occurred on cellulose were sampled, cultured and identified by sequencing the ITS1-5.8S-ITS2 regions of their rDNA. Six celluloytic taxa with high isolation frequency were found to represent the fungal community in the first phase of cellulose colonization. Functional diversity of individual fungal isolates was analyzed using the carbon source phenotype microarray technique so as to generate a detailed profile of their functional capabilities. The measurement of the overlap in the resource utilization of species was determined. The same overlap index was used to measure the co-occurrence of species on the cellulose. At an early stage of cellulose colonization, a clear potential overlap in the overall metabolic functions of the pioneer species was present. Interestingly, the species that prevailed at the beginning of colonization did not overlap each other in the field sites. The presence of different pioneer fungal species which can utilize plain cellulose in a given environment represents a guarantee for the "ecosystem services", and the fact that their distribution is patchy is in accordance with the classical ecological rule as regards species exclusion, although it seems that this can be applied for fungi on a very small scale. The results of the study provided evidence of competitive exclusion as a determinant of fungal diversity, and gave chances to the neutral theory predicting that species assemblages are structured by stochastic processes such as initial dispersal into an area, or founder effects.

Combining metagenomics and cultivation approaches to investigate the yeast community of forest soil

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Even though yeasts represent an important part of soil microbial communities, our current understanding to their ecology is insufficient. Different environmental distribution of yeasts and filamentous fungi is a consequence of specific phenotypic traits, such as the unicellular versus filamentous growth, which leads to different dispersal and colonization abilities. Therefore, specific focus on yeast communities has been put on during this study.
Series of field sampling of soil and litter over 70 km2 from forest soils of the temperate zone (Czech Republic, Central Europe) was performed to characterize the communities of fungi and yeasts. The sites differed with regard to dominant tree (spruce, beech, oak and mixed forests) as well as to the soil chemistry (alkaline to acidic pH, organic matter content). DNA was extracted and the ITS2 was amplicon-sequenced on Illumina Miseq. Sequences belonging to yeast species were selected and further analyzed.
In parallel yeast strains were isolated from soil and litter at these sites. The sequences of the ITS2 region of the cultivated isolates were compared with environmental sequences in order to answer questions regarding the representativeness of cultivated species as compared with the yeast community assessed with metagenomics. The cultivation approach yielded several strains that belonged, according to the analysis of environmental DNA, to the most abundant yeast species.
Interestingly, some of the most dominant species assessed with environmental DNA appear to represent undescribed species for which we intend to provide the correspondent formal description in the near future.

Isolation and cultivation of actinobacteria from acid soils with high occurrence of Trebon clade

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The Trebon clade of actinobacteria is expected to participate in decomposing activities in acidic soils as it dominates the actinobacterial clone libraries from acidic soil of waterlogged deciduous forest near Trebon and acidic soil under coniferous forest near Zakopana in the Czech Republic. The purpose of the current study was to develop media and conditions for isolation of the Trebon clade representatives.
Mineral and organic horizons of acid waterlogged Trebon soil (gleysol by FAO classification) with average pH 3.5 and 4, respectively, were chosen for isolation of actinobacteria. Four different media were used for growth of bacteria from soil suspension: mineral agar G-1 for selective isolation of actinomycetes, organic medium R2A, humic acid-vitamin agar HV for selective isolation of rare actinomycetes and acid medium VL55 containing xylan, on which a representative of Trebon clade was isolated in previous studies. Different incubation periods from 1 to 4 weeks and different temperatures were used. After isolation only gram-positive bacteria by (KOH test) were selected. PCR and consequent sequencing of 16S rRNA with universal primers were performed. Raw taxonomic assignments were done with RDP Classifier tool, confirmed by BLAST.
1518 bacteria were isolated. After elimination of gram-negative bacteria and all morphologically apparent *Bacillus* sp., 496 gram-positive bacteria were chosen for the sequencing. Sequence of 99 strains showed 58% of strains to be *Streptomyces* sp., 20% *Streptacidiphilus* sp., 6% *Kitasatospora* sp., 3% *Actinospica* sp., 3% *Nocardia* sp., 2% *Catenulispora* sp. and 7% non-target bacteria *Bacillus* sp.
The preliminary results demonstrated that even though more than half of the sequenced isolates were streptomycetes common for any soil, quite high percentage of representatives belonged to *Streptacidiphilus* sp., a genus common for acid environments. All representatives of so-called rare actinomycetes (non-streptomycetes) were isolated on VL55 medium, which makes it potentially more preferable for isolation of the target Trebon clade. Actinobacteria selected on VL55 medium are supposed to be interesting objects for studies of bacterial interactions, as they grew in close association with gram-negative bacteria on the same medium and could be separated only on a rich medium, allowing faster growth for their gram-negative neighbors.

Fire recurrence effects over the structure and activity of ectomycorrhizal fungal communities in Mediterranean pine forests

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Fire is an intrinsic factor of Mediterranean forest ecosystems, determinant of abiotic disturbance and organism's ecology. Wildfire recurrence and predicted increase associated with global change may particularly alter the fertility and microbial communities of soil. Symbiotic ectomycorrhizal (EM) fungi are main actors of forest soils, directly involved in the cycling of nutrients and the productivity of trees.
We surveyed forests of two representative Mediterranean pines, *Pinus pinaster* Ait. (Ppi) and *Pinus halepensis* Mill. (Pha), to evaluate whether fire recurrence affected the structure and functioning of EM fungal communities. Ectomycorrhizal root tips of both pine species were collected in low and high fire recurrence forest sites, measured for enzymatic activities (EAs) involved in geochemistry cycles and nutrient mobilization, and fungal identification approached by high-throughput sequencing.
Fire recurrence significantly altered the assemblage of EM fungi, while the community richness remained unaffected. EAs secreted by EM fungi highly diverged depending on the tree species, being generally greater in the rhizosphere of Pha compared with that of Ppi. High fire recurrence negatively impacted the activity of the Ppi ectomycorrhizas, whereas in the case of Pha, most EAs were unaffected. A general increase of laccase activity, involved in the oxidation of various substrates, such as phenols or lignin, was observed in recurrently burned areas. Fire recurrence effects might be explained through the divergent altered edaphic properties and specific fungal community composition of the forests studied, which could have relevant implications on decomposition rates, carbon storage, and availability of nutrients in soils, finally affecting the productivity of Mediterranean forest ecosystems.

High richness of ectomycorrhizal fungi and low host specificity in a coastal sand dune ecosystem

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Ectomycorrhizal (EM) fungi are ubiquitous in temperate and boreal forests, and comprise over 20,000 species forming root symbiotic associations with Pinaceae and woody angiosperms. Several tens or more of different EM fungal species can coexist and interact within the same tree species, forming complex multispecies networks in soils. The structural properties of these interaction networks (e.g. nestedness and modularity) may determine plant and fungal community assembly and species coexistence, yet their structure has been little studied in northern coniferous forests where trees depend on EM fungi for nutrient acquisition. We used high-throughput sequencing to extensively describe bulk soil and root-associated fungal communities in four co-occuring Pinaceae in a relic foredune plain located at Îles de la Madeleine, Qc. We found high EM richness, with a total of 200 EM operational taxonomic units (OTUs) across the four different hosts, mainly belonging to the Agaricomycetes. Network analysis did not reveal significant nestedness. On the contrary, we observed anti-nested patterns in both bulk soil and roots EM fungal communities. However, we could not define significant modules (i.e. subgroups interacting species) which indicates low level of specialization in host-EM fungi associations. We did not observe clear patterns in OTU richness or in community structure as described with ordination analysis. Despite the low level of host preference observed, many less frequent OTU appeared to show some degree of specialization to a given host and were able to identify both generalist and specialist OTUs. Finally, this study revealed no host effect on the structure of EM communities and, as a first comprehensive analysis of EM interactions in a coastal dune forest, brings a new level of understanding of the architecture of tree-EM fungal species interaction networks.

Revealing sources of biological methane production in boreal upland forest

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Boreal upland forests are considered as a sink for the greenhouse gas methane (CH_4) due to methanotrophic microbes that oxidize CH_4 in soils. Recently, several studies have confirmed that emissions of CH_4 from vegetation can occasionally overcome the sink strength of the soil, and the forest ecosystems may in total act as a source of CH_4. However, the origin and the production mechanisms of CH_4 emitted from trees still remain controversial. Especially it is unclear whether the plant-emitted CH_4 originates from biotic or abiotic processes within the forest ecosystem. Our aim was therefore to assess whether methane producing microbes (methanogens) could account for CH_4 emissions within a boreal forest.
To detect the abundance of the methanogenic community, samples of the most prevalent plant and tree species and soil, peat and decayed wood were taken in June 2014 and 2015 from the Scots pine (*Pinus sylvestris* L.) dominated forest at the SMEAR II station (Station for Measuring Ecosystem-Atmosphere Relations) in Finland. Five replicate samples from each material were divided into different compartments: shoots, stem and roots, or upper and lower layer of soil and peat. DNA was extracted and qPCR analysis of the *mcrA*-gene was performed to quantify the methanogenic community. To link the presence of the *mcrA*-genes to the CH_4 exchange, the CH_4 fluxes were measured from different compartments of the forest (forest floor, tree stems and shoots) during 2013–2015.
Based on our 3-year CH_4 flux measurements, the forest floor acted as a sink of CH_4 for most of the year; however, some emissions occurred mostly from the wet spots of the forest from May to July every year. Tree stems and shoots emitted small amounts of CH_4 throughout the year, with the highest emission rates from trees growing on the wet locations. From the wet spots of the forest floor, high number of the *mcrA*-gene copies were detected, while the *mcrA*-gene copy number from drier mineral soil samples were under the detection limit. The qPCR analysis is still ongoing, but our preliminary results indicate that the *mcrA*-gene copies can also be detected from the understory vegetation, e.g. different mosses and roots of *Equisetum sylvaticum*. These preliminary findings seem to support our hypothesis that methanogens are involved in the CH_4 production in boreal forest ecosystems, and that they can be detected and quantified from the soil and in vegetation.

The effect of photosynthesis-derived C flow on the microbial community structure and enzymatic activities in boreal forest

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The boreal forest soils are globally significant reservoirs of carbon(C). In the organic layer of boreal forest soil, humic compounds form the most stable C pool. In these soils, growth limiting nutrient is often nitrogen(N) that is tightly incorporated into soil organic matter(SOM). Therefore, the release of N from SOM is a key factor regulating the productivity of boreal forest ecosystems.
When the amount of atmospheric CO_2 increases, it accelerates the plant photosynthesis rate and increases the C flow to the soil. Recently, it has been shown that the plant-derived, easily utilizable, C accelerates the decomposition of the older and more slowly degradable SOM, i.e. humus, through a phenomenon called the priming effect. It has also been suggested that the availability of N is one of the main factors affecting the rate of priming. Moreover, the ectomycorrhizal fungi, which get most of their carbohydrates from the host plants, have been shown to be involved in the SOM decomposition and priming.
The focus of this work was to study the effect of plant-derived C on the structure and enzymatic activities of the microbial community in boreal forest humus. For that, a three-year field experiment was set up at the Hyytiälä forestry field station in southern Finland. In the experiment, bags(24 per treatment) filled with sieved(4mm) humus were buried between organic and mineral soil horizons. The below-ground C fluxes were controlled with three different mesh sizes of the bags, 1µm, 50µm and 1mm. The different mesh sizes prevent the entrance of plant-derived C into the bags, exclude root-derived C from the bags but allow fungal hyphae to penetrate, or allow penetration of both fungal hyphae and fine roots, respectively.
Enzymes were recovered from humus bags by filter centrifugation. Enzyme activities of acid phosphatase, N-acetylglucosaminidase, b-glucosidase, b-glucuronidase, b-xylosidase, cellobiohydrolase, and leucine amino peptidase were measured with fluorometric assay. Laccase activities were measured with colorimetric assay. The changes of the microbial community structure in the humus bags were observed with the phospholipid fatty-acid analysis(PLFA). The C and N contents and mass losses were analyzed.
The preliminary results indicate that the plant-derived carbon affects both on the structure of soils fungal community and its enzymatic activities. The results will be discussed in relation to the effect of photosynthesis-driven C and SOM decomposition.

Archaeal, bacterial and fungal abundance and diversity along an altitudinal gradient in Alpine forest soils

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Altitudinal mountain gradients are characterized by dramatic changes in biotic and abiotic conditions over short geographic distances and have been studied to know the effect of environmental factors on biota. However, the knowledge about soil microbial communities' patterns over altitudinal gradients in zones especially vulnerable to global climate change, such as European Alps, is still scarce. Here, we investigated four forest sites in the Italian Alps over an altitudinal gradient from 545 to 2000 m a.s.l. These sites represented a climosequence including submontane, montane, subalpine and alpine vegetation zones. Microbial communities in soils from the four sites were characterized with regard to archaeal, bacterial and fungal abundance as well as diversity, using qPCR and Illumina sequencing, respectively. Soil physicochemical properties were also determined.
The relative size of the archaeal community did not significantly vary along altitudinal gradient and its phylogenetic composition was dominated by Thaumarchaeota. The relative bacterial abundance increased with altitude, which was related to increasing levels of organic matter and nutrients with altitude. The dominant bacterial phyla were Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes. The submontane site harboured the highest bacterial richness and diversity, however, a significant monotonic decrease of richness and diversity properties of the bacterial community with altitude was not detected. The bacterial community structure significantly differed between sites, although the montane and the alpine sites contained similar bacterial communities, which was related to the similar pH at both sites. pH was the best predictor of variation in bacterial community structure and diversity. The relative abundance of the fungal community also increased with altitude and its phylogenetic composition comprised Ascomycota, Basidiomycota and Zygomycota. A clear trend of fungal diversity along altitude was not found and the structure of the fungal community significantly differed between the four sites. Correlation analyses demonstrated that pH and C/N were the main abiotic factors governing changes in fungal richness/diversity and community structure, respectively. The variation of the predominant bacterial and fungal classes over the altitude gradient was the result of a complex interaction between the ecological roles of each class and the environmental factors prevailing at each site.

Impact of wild boar (*Sus scrofa*) rooting on the soil seed bank in Białowieża Forest

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Animal activity can stimulate germination of seeds in soil seed banks by creating gaps in the compact plant cover. The aim of this study was to assess the impact of wild boar rooting on the soil seed bank in a natural oak-linden-hornbeam forest. Observations were carried out on permanent plots (100 squares of 8 m x 8 m) in the strictly protected zone of Białowieża National Park. Samples of the soil seed bank were taken from plots which showed varying intensities of wild boar rooting (frequency and average percentage of exposed ground surface). Areas to be sampled were selected on the basis of archival data from the years 1975-85 and 1991-2007. Squares were divided into: low (series A), medium (series B) and highly rooted (series C). The series of squares significantly ($P<0,001$) differed in their intensity of rooting. Research was conducted by the seedling emergence method during two vegetation seasons. Alltogether, 7985 seedlings (of 67 taxa) germinated from 240 soil samples. The highest number of germinated seedlings and species were found in the highly rooted squares. In all the series dominant species was *Urtica dioica*. In the soil seed bank 19 species of anemochory germinated, and accounted for 27.5% of all species present in the seed bank of all series. Permanent wild boar rooting increased the species richness in the soil seed bank and increased the amount of species with higher light requirements.

Spatial heterogeneity of mountainous soil is associated with high beta diversity of microbial community

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Microorganisms play an irreplaceable role in ecosystem functioning and despite recent progress, the understanding of the spatial diversity of their community, its drivers and consequences for ecosystem functioning is limited. We carried out a small-scale survey of a 36 m2 site of mountainous soil in the Bohemian Forest (Czech Republic, central Europe) covered by patchy, spatially variable ground vegetation to determine the factors that drive microbial community composition and to quantify the α- and β-diversity of the community. In total, 48 soil cores (4.5 cm) were collected in June 2011. Dominant understorey vegetation near cores was recorded and the litter and organic soil horizon chemistry (total and available C, N, P, and total phenolics) was characterized. Fungal and bacterial biomass and community composition were estimated by qPCR and Illumina MiSeq sequencing of ITS2 and 16S rDNA, respectively. Activity of several extracellular enzymes was also measured.
Alfa-diversity in all samples calculated on random selection of 1000 sequences per sample was high with 4675 bacterial and 3568 fungal OTUs, with per sample average 357 bacterial and 173 fungal OTUs. Alfa-diversity of OTUs with relative abundance >1% was higher in fungi (219 OTUs) than in bacteria (121 OTUs) and was higher in litter than in soil. Whittaker´s beta-diversity index was higher in both horizons for fungal compared to bacterial OTUs in the whole community and did not reach saturation even when all 48 samples were included. Only when considering OTUs with relative abundance >1% the beta-diversity of bacterial OTUs saturated after 10 samples while to cover majority of different fungal OTUs over 40 samples were needed. Community composition was significantly different between L and S horizons for both fungi bacteria, but compared to bacteria fungal community composition greatly varied among samples within a horizon on various taxonomic levels. There was strong correlation of vegetation cover and fungal community composition to geographical distance. Bacterial community was correlated to geographical distance only in litter horizon. The relationships between microbial community composition and measured biotic and abiotic factors were complex, but ground vegetation seemed to have stronger influence then soil chemistry, especially in litter. Enzyme activity was more related to soil chemistry in soil horizon while in litter horizon was more related to the composition of the fungal community.

Mycorrhizal community structure across an alpine tree line ecotone

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The tree line is an important ecotone of the elevation gradient which is expected to be significantly affected by climate change. Mycorrhizal fungi can influence tree fitness under suboptimal conditions, because they provide the trees with nutrients in exchange for carbon and possibly interconnect different species via a common mycelial network. Six elevation gradients of 4-5 plots across an ecotone formed by *Picea abies* and *Pinus mugo* were established in the Krkonoše Mts (Czech Republic) to study mycorrhizal community structure. In order to use historical data on the occurrence of fungal fruit bodies at the tree line, find differences between various methods and describe the life strategies of the dominant species, we conducted 454 pyrosequencing of soil fungal DNA, estimated mycelial biomass in meshbags, identified fungal symbionts in ectomycorrhizae and counted fungal fruit bodies.
In total, 5-19 operational taxonomic units (OTUs) of mycorrhizal (predominantly ectomycorrhizal) fungi with higher abundance than 0.5 % of reads per plot were detected. Mycorrhizal OTU composition was best explained by tree height and reflected stand type differences along the elevation gradients. At *Picea abies* sites, *Tylospora fibrillosa, Russula ochroleuca* and *T. asterophora* were the most abundant OTUs; the same species were most often identified in ectomycorrhizae. *R. ochroleuca* produced also the most fruit bodies, whereas those of *Tylospora* were rarely found. The most abundant OTUs at *Pinus mugo* shrubs were *Amanita submembranacea, Piloderma sphaerosporum* and *T. fibrillosa*; the latter two species were most frequently detected in ectomycorrhizae together with *Suillus variegatus*. Most of the fruit bodies were produced by *Lactarius rufus* and *S. variegatus*. Biomass of ectomycorrhizal fungal mycelia in mesh bags and the number of dominant mycorrhizal OTUs, representing more than 5% of reads per plot, decreased towards the top of the gradient.

Patterns of soil fungal communities in subtropical Chinese forests in relation to plant diversity

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Soil fungi play a key role in terrestrial ecosystems. A vast diversity of saprotophs and mycorrhiza contribute to plant development through efficient decomposition and nutrient uptake, respectively. However, the impact of plant diversity on the fungal community composition is not resolved and especially unclear in the subtropics.
The world´s largest biodiversity and ecosystem functioning experiment in forest (www.bef-china.de) is located in southeast China and constitutes the ideal project to study the soil fungal community in a subtropical forest ecosystem. Within the experiment two sites were planted 2009/2010 with a total of 42 tree species being equally ectomycorrhizal or arbuscular mycorrhizal. A tree diversity gradient with five diversity levels (1, 2, 4, 8, 16) was planted at both sites. In October 2011, one site was sampled and DNA was extracted from soil cores.The ITS region was amplified and sequenced by 454 GS FLX+ technology. This provided a deep-resolution overview of the main fungal functional groups namely saprotrophic, pathogenic, arbuscular mycorrhiza and ectomycorrhiza fungi for every diversity level and tree species. The evaluation of the sequencing data included the correlation with a broad range of metadata. The main drivers for each of the fungal functional groups will be identified for this early stage of forest development. This study sets the baseline for the long-term investigation of general and mycorrhizal fungi in subtropical forest ecosystems.

The yield of onions and its quality depending on mycorrhiza inoculation

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The positive effect of mycorrhiza fungi on the onion yield is well known, but there is less research done about the impact of these microorganisms on biochemical composition of experimental plants. Experiments were carried out with onions cv 'Exibition' at Latvia agro-climatic conditions in peat and sandy soils. Total content of phenols and flavonoids, and antiradical activity were determined spectrometrically. Ascorbic acid content was determined titrimetrically with 2,6-dichlorophenol-indophenol.
Mycorrhiza treatment resulted in changes of onion yield that varied between -8% and 28% in comparison with untreated plants.
Results showed that mycorrhiza fungi caused differences in biochemical composition of onions. Ascorbic acid content increased by 0-23%, total phenol content by 2-45 %, differences in antiradical activity varied from -19% till 36% in comparison with untreated plants. The effect of mycorrhization on onion biochemical content depended on soil properties and onion development stage.

Earthworm-microbe interaction can be associated to less harsh conditions in green sugarcane systems

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Earthworms are known as ecosystems engineers and bioindicators for soil quality. Their improvements on agricultural soils can be extended to benefits on plant growth. However, soil microbiome response in this context is a question of concern. Burrow walls (< 2 mm layer surrounding burrows), casts and guts are a hotspot for microbial activity. Recent evidence suggests that this so called drilosphere (i.e., earthworm impacted soil) might extend further into the soil. Thus, we analyzed microbiome response to earthworm activities in soil previously regarded as non-drilosphere soil from a macrocosm experiment simulating sugarcane production with and without earthworms (E), with and without straw (S) and mixing both factors (ES). We applied quantitative PCR to determine the total abundance of Bacteria and Archaea related 16S rRNA genes and whole community shotgun sequencing (Illumina MiSeq) to analyze class-level community composition. Bulk and rhizosphere soil were included in the soil sampling, drilosphere was not analyzed. In E pots, growth of Bacteria was stimulated in bulk soils, while abundance of Archaea was reduced in ES rhizospheric soils. In parts, comparing to the Control group, microbiome response activated in E was the same than in S: in bulk soils a lower abundance of Actinobacteria and Delproteobacteria and a higher abundance of Bacilli were observed, while in rhizosphere a higher abundance of Alphaproteobacteria was observed. All the treatments (E, S and ES) improved plant growth. In our experiment, earthworm impacted soil microbiome responses that could be associated to less harsh conditions in sugarcane crop systems, supporting the view that the earthworm impact on soil microbiota extends beyond the drilosphere sensu stricto.

Carbon source and availability influence the production of antimicrobial compounds

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A soil is considered disease-suppressive, even in the presence of the pathogenic agent in the soil community, when the plant does not show symptoms of pathogenicity.
Pseudomonas spp. and *Streptomyces* spp. are frequently found in high quantities in suppressive soils, and may have key functions in determining their pathogenic suppressive soil characteristic.
Apart from the biotic factors (the microorganisms), the diversity and availability of nutrients have been referred as determinants in the occurrence and function of soil microorganisms, Pseudomonas spp. and Streptomyces spp.
The aim of this study was to verify if different carbon sources - glucose, saccharose, mannitol, maltose and xylose - influence the production of antimicrobial compounds by microorganisms and if different concentrations (1%; 0.4% and 0.04%) of those carbon sources would contribute to the expression of new phenotypes by *Streptomyces* sp. and *Pseudomonas* spp.
Co-culture interactions were conducted between putative antimicrobial producer and indicator strains (including bacteria and fungus) in solid media culture with the distinct carbon sources and concentrations.
The source and concentration of carbon influenced the interaction between putative antimicrobial producers and the indicator strains. Most of the inhibitions observed were dependent on the interaction of the three factors: carbon source, carbon concentration and the putative antimicrobial producer.
In conclusion, the source of carbon and the correspondent concentration are abiotic factors that mediated the production of antimicrobial metabolites by certain bacteria that are usually present in high levels in suppressive soils, and then these soils can act as disease control method.

Rhizosphere of olive tree: a source of plant growth promoting bacteria

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Plant Growth-Promoting Bacteria (PGPB) are associated with many plant species and commonly present plant microbiota. The most widely studied group of PGPB are plant Growth Promoting Rhizobacteria (PGPR) which colonizes the root surfaces and the plant rhizosphere. Countles free-living PGPR such as *Pseudomonas, Bacillus, Arthrobacter* etc. have used promote plant growth and control phytopathogens in agricultural fields. The widely recognized mechanisms of biocontrol mediated by PGPB are competition for an ecological niche or a substrate, production of inhibitory chemicals, and induction of systemic resistance in host plants to a broad spectrum of pathogens. Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture.
There has been many studies describing potential uses of plant associated bacteria as agents stimulating plant growth and managing soil and plant health.
In this study bacteria were isolated from rhizosphere and soil surface of one olive tree and one olive sapling. The capacity for plant growth promoting activities and antagonistic features were tested by using siderophore production test, HCN production test, phosphate solubilization test, indole-3-acetic acid test, amonnium production test, antibacterial and antifungal activity test. The isolates showing at least 3 out 4 namely phosphate solubilization, indole-3- acetic acid production, and siderophore productions assumed to be PGPR and they were identified by using fatty acid methyl ester (FAME) analysis and automated Ribotyping. The bacteria were defined as *Flavimonas oryzihabitans, Pseudomonas putida* biotype A *Acinetobacter haemolyticus, Rhizobium radiobacter, Bacillus atrophaeus, Bacillus subtilis, Bacillus megaterium* and *Arthrobacter globiformis*.

The effects of michorrhyza fungy on the tomatoe plant water retention ability

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Arbuscular mycorrhizal fungi (AMF) are symbiotic soil fungi that colonize roots of the majority of vascular plants. Therefore fungi are important in agriculture, because it improve plant water relations, increase mineral uptake, which reduces the use of fertilizers.
The aim of study is to access the influence of arbuscular mycorrhizal fungi on the growth and water retention ability of tomatoes. The cultivars 'Zyska', and 'Jantar' were grown from seeds. Fungus preparation was added under seeds during sowing. Control variant - without inoculation with mycorrhizal fungi. At the 3rd true leaf stage seedlings were planted in open field conditions. Fresh and dry weight was detected during plant growth. Water retention ability was detected gravimetricaly twice during plant growth. Results showed that mycorrhiza fungi stimulated seedling and plant growth. Water retention ability was increased as result of mycorrhization. Differences between varieties was detected.

Characterization of plant-associated bacteria isolated from highly drought tolerant Pistacia therebinthus

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Biotic and abiotic stresses affect the growth of plants all over the world. Among them, drought is a major problem since water is essential for all physiological processes of the plant. Water shortage inhibits plant growth and leads to reduced biomass production. Many plant-associated bacteria (rhizosphere bacteria and endophytes) possess several direct and indirect mechanisms to promote plant growth. These mechanisms include provision of nutrients such as fixed nitrogen, iron and phosphorus; production of phytohormones such as indole-3-acetetic acid (IAA); production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which is responsible for the decrease of the stress hormone ethylene; protection against toxic metals and other stressors and inhibition of various pathogenic agents. Bacterial excretion of exopolysaccharides (EPS) acts as a boundary between cells and surrounding environment, protecting them against environmental stress. Biofilm formation seems to protect bacterial cells from hostile conditions and helps plants by regulating the flow of water and nutrients across the roots. Also bacteria producing osmoprotectans (such as proline) possess the capability to facilitate plant growth under drought stress.
Plant-associated bacteria were isolated from *Pistacia therebinthus* trees from an arid region in Bulgaria. The cultivable bacterial population from leaf, stem, root, rhizosphere soil and bulk soil samples were phenotypically and genotypically characterized. Phenotypical characterization consisted of two parts – plant growth promotion tests and drought tolerance tests. 211 isolates were tested for production of IAA, ACC-deaminase, siderophores (which help for the uptake of iron), fixation of nitrogen and solubilisation of phosphates. 25 strains showed positive results for all 5 tests and were further investigated for the production of EPS and proline. Whereas all of them were capable to produce EPS and proline, all 25 isolates were genotypically characterized by sequencing of the 16S rDNA. The results of the sequencing show that the 25 most promising strains belong to 8 genera, but most represented are *Pseudomonas, Pantoea* and *Arthrobacter*. A few consortia of endophytic and rhizosphere bacteria were used in an inoculation experiment to evaluate whether the in vitro plant growth promoting traits are occurring in planta as well. The latter experiment was performed on grass in climate chambers where all conditions are controlled.

Antibiotic activity of actinobacteria associated with millipedes and earthworms

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One of the problems in the microbiology and medical sciences is the multidrug resistance of bacteria and fungi to antibiotics. Therefore, the necessity of finding new antibiotics producers capable to inhibit soil microorganisms among which there are potential pathogens for animal and human. For tests, we used pure test-cultures of actinobacteria Streptomyces, 40 strains of 13 species, isolated from associations with soil millipedes, *Cylindroiulus caeruleocinctus* (Wood), *Pachyiulus flavipes* (C.L. Koch), and the earthworm *Eisenia fetida andrei* (Sav.). Antibiotic activities of the isolates were tested toward opportunistic pathogenic test-organisms *Aspergillus niger* INA 00760, *Candida albicans* ATCC 2091 and *Bacillus subtilis* ATCC 6633.
Pure isolates of streptomycetes obtained from the preliminary selection were cultivated on Gauze-agar. After incubation at 28°C for 10 days, these isolates were subjected to screening via agar overlay technique by placing agar blocks on the growing test-microorganisms. The plates were incubated at 18-20°C for 4 days and zones of inhibition were measured periodically.
Fifty % of the actinobacteria strains exhibited high suppressive activity, three of them showed the inhibition zone of 15-25 mm in diameter with all the test-organisms. Seven strains were active against *A. niger* and *C. albicans*. Four strains inhibited *B. subtilis* and *C. albicans* only. The most active streptomycetes were the strains isolated from the gut of *C. caeruleocinctus*.
The selected strains were additionally tested to reveal the mechanisms and spectrum of their activity. For this purpose the panel of tests, including growth and proliferation inhibition, translational and specific protein interaction kinetics were utilized. The cultural medium was extracted afterwards by using the common procedures of liquid-liquid extraction and chromatographic separation. The isolated fractions were subsequently tested to determine the ones containing the active substances. The “active” fractions were concentrated and left at the freezer for further tests and identification. The actinomycetes can be used as producers of new biologically active substances, in particular, new antibiotic.
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Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on N_2O emissions from clover residues and interaction with the earthworm *Lumbricus terrestris*

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Mineralization and nitrification of residue N stimulate N_2O emissions after grassland cultivation. As a novel application, we simulated spraying of DMPP on clover pasture before cultivation to delay nitrification and residue-induced denitrification. Insofar as the anecic earthworm *L. terrestris* contributes to the decomposition of incorporated residues, this could be influenced by DMPP by changing earthworm feeding behavior. Furthermore, nitrate reduction in the earthworm gut is a potential source of N_2O that could also be affected by DMPP presumably by reducing nitrate intake and denitrifiers. In this mesocosm study *L. terrestris* (141 m-2) were incubated in a sandy loam grassland soil with ^{15}N-enriched (0.94, 0.88 and 0.77 ^{15}N-atom% in leaf, stolon and roots) clover residues where above-ground parts were treated with 1 kg ha-1 DMPP (+DP) or left untreated. Residues were either buried at 10 cm depth to simulate grassland ploughing (PL) or mixed with the soil at 0-10 cm depth to simulate rotovation. The N_2O emissions during 28 d were reduced by 40 and 9% by DMPP in PL and RO, respectively. Then earthworms were recovered (90% survival) and individual worms (twelve per treatment) were incubated to determine in vivo N_2O emissions, which were 0.26±0.28 (PL), 0.10±0.10 (PL+DP), 0.31±0.29 (RO) and 0.15±0.11 (RO+DP) nmol N_2O g (fresh wt.)-1 h-1. These rates were low compared to N_2O emissions from intact mesocosms. The large variability was probably due to variation in time since last meal. Earthworms in DMPP treatments tended to produce less N_2O, but a significant difference ($p<0.05$) was only found between RO and PL+DP. These earthworms were then dissected to remove the gut and the remaining tissues were freeze-dried for ^{15}N analysis to determine their involvement in residue turnover. Worms incubated with residues had significantly ($p<0.05$) higher ^{15}N-atom %, 0.3989 (RO), 0.4063 (RO+DP), 0.4427 (PL) and 0.4793 (PL+DP) than those from residue-free soil (0.3684). There was no significant effect of DMPP, hence no evidence for negative effects on feeding behavior of *L. terrestris*. In summary, interactions between *L. terrestris* and nitrifier-denitrifier activity were shown. The trend of less N_2O from earthworms in DMPP treatments, a trend also seen for intact mesocosms, could be due to 1) lower nitrate concentration in ingested residues and associated soil, and 2) lower abundance of denitrifiers in ingested soil, as reflected by lower N_2O emission from intact mesocosm.

Suppressive activity of the intestinal fluid of diplopods against yeasts

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For the tests, we used pure test-cultures of yeast isolated from associations with soil millipedes, *Cylindroiulus caeruleocinctus* (Wood), *Pachyiulus flavipes* (C.L. Koch) and other habitat. Also we had different strains of the same species.
We have taked new experiment: the growth of colonies of yeast after short term impact ot the intestinal fluid from middle and rear section of diplopoda's intestine. Different reactions of yeasts have been found.
The difference in reactions does not depend on the habitat yeast.
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Kin discrimination between sympatric soil isolates of *Bacillus subtilis*

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Microorganisms are directly influenced by actions of their neighbors and cooperative behaviors are favoured among relatives. Only a few microbial species are known to discriminate between kin and non-kin and distribution of this trait within sympatric bacterial populations is still poorly understood. Here we provide evidence of kin discrimination among micrometer scale soil isolates of *Bacillus subtilis*, which is reflected in striking boundaries between non-kin sympatric conspecifics during cooperative swarming on agar. Swarming incompatibilities were frequent and correlated with phylogenetic relatedness, as only the most related strains merged swarms. Moreover, mixing of strains during colonization of a plant root suggested possible antagonism between non-kin. The work sheds light on kin discrimination of a model Gram (+) bacterium and suggests a potential effect of this fascinating mechanism on ecological interactions in soils and rhizosphere.

Stefanic et al. Kin discrimination between sympatric *Bacillus subtilis* isolates.PNAS, 2015, in press doi:10.1073/pnas.1512671112.

The role of rhizosphere microbiome in soilborne fungal disease suppression in common bean

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The rhizosphere is an ecological niche that shapes the inhabitant microbial community structure. Many beneficial microorganisms in the rhizosphere provide plants with mineral nutrients, phytohormones, and also help to protect the plant against soilborne pathogens. The characterization of the microbial communities and their functional potential in rhizosphere is important to link microbial activity and plant health. Here, we aimed to identify potential microbial groups and functional traits correlated to the suppression of the soil borne pathogen *Fusarium oxysporum*, the causal agent of Fusarium wilt on common bean. We used shotgun metagenomic sequencing to investigate the rhizosphere microbiome of two common bean cultivars classified as resistant (Mil?nio) and susceptible (Alvorada) to Fusarium wilt. Plants were grown in mesocosms experiments in Amazon Dark Earth, a soil with high microbial diversity. Chemical analysis indicated a significant increase of pH, Ca, Fe, Zn, Mn, B, nitrate, cation exchange capacity, sum of bases and base saturation in rhizosphere of both common bean types. The microbial community structure of rhizosphere, classified at genus level, was different from the bulk soil, revealing the selection process in this environment. Quantitative PCR showed higher 16S rRNA copy number in the rhizosphere of the resistant common bean when compared with susceptible material. The resistant cultivar presented higher taxonomic diversity but lower functional diversity. The most abundant phyla in rhizosphere were Proteobacteria (41%), Actinobacteria (31%), Firmicutes (5%), Acidobacteria (3%) and Chloroflexi (3%). The resistant cultivar presented higher abundance of the phyla Chlamydiae, Spirochaetes, Deinococcus-Thermus and Chrysiogenetes in comparison to the susceptible cultivar and bulk soil. Comparing the microbiome composition of resistant and susceptible cultivars in a deeper taxonomic level, 24 genera presented higher abundance in the resistant one, highlighting *Bacillus* and *Pseudomonas*. Preliminary analysis showed that there is a specific selection of the microbial communities inhabiting the rhizosphere of a resistant common bean cultivar. Further analysis will combine 16S rRNA gene sequencing and metatranscriptome for a deep taxonomical and functional analysis. FAPESP 2014/03217-3 2015/00251-9

Decay in the canopy

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The effort to describe assemblages of wood rotting fungi have focused almost exclusively on communities within substrata on the woodland floor yet the process of community development often starts in the canopy in branches still attached to standing trees. Due to priority effects, the identity of wood decay fungi that establish during these early stages of community development affects saprotrophic community composition once the substrate reaches the woodland floor however little attention has been paid to priority effects from the perspective of early-stage decayers. Abiotic stressors such as low oxygen availability, transportation of water by healthy sapwood and production of inhibitory extractives by the host are hypothesised to be significant determinants of community development in attached branches. Conversely biotic stress, largely in the form of interspecific competition for space, is considered to become increasingly important as decay advances and abiotic conditions ameliorate.
We are employing a combination of traditional culturing methods, DNA sequencing and modelling to map communities of wood rotting fungi in branches attached to standing and windthrown trees. The relative importance of abiotic and biotic stress factors in naturally occurring communities will be investigated by applying a proteomics to investigate the stress responses of resident organisms. This will be the first time such an approach will have been applied to such recalcitrant substrata and our data will contribute to bridging the gap between observational and experimental approaches; a fundamental challenge both in terms of understanding decay communities and in the wider ecological context.

Microbial airborne talk: effect of fungal volatiles on bacteria

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In terrestrial ecosystems fungi and bacteria live in close proximity and can form complex, multi-species networks. Within those networks, both fungi and bacteria produce a plethora of secondary metabolites representing diverse chemical classes. Recent studies have shown that volatile compounds play important roles in microbial interactions, both as signaling compounds and antimicrobials (Garbeva et al., 2014a;b;Schmidt et al., 2015). However, little is known about the role of these compounds in the communication between fungi and bacteria. Here, we exposed different soil bacteria to volatiles emitted by soil fungi and oomycete. Metabolomics analysis revealed different volatile patterns for all fungi and oomycete tested. Furthermore bacterial strain specific phenotypic responses were observed depending of the fungal volatile patterns. Two bacteria namely Collimonas pratensis and Serratia plymuthica showed the strongest response to fungal volatiles by increased growth and motility.
Here we provide new insights into volatile mediated fungal-bacterial communication. To gain a deeper understanding of the underlying mechanisms of how volatiles are perceived by bacteria, we are currently investigating the interaction between Fusarium culmorum and Serratia plymutica on transcriptomics, proteomics and metabolomics level.

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Specificity and biotic local selection in *Streptomyces* interactions influences antimicrobial activity

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The rhizosphere has an extremely diverse microbiome which is characterized by a dynamic net of interactions among microorganisms and between them and plant roots.
Within the rhizospheric microbial communities a high abundance of *Streptomyces* strains is commonly found. This group of bacteria have the ability to produce a wide variety of secondary metabolites with antimicrobial activity, which play an important role in the protection of plants against pathogenic agents.
The aim of this study was to evaluate (1) whether specificity in the interaction among *Streptomyces* strains growing in co-culture influences the antimicrobial activity against other soil bacteria, including phytopathogenic ones; and (2) if the frequency of antagonistic phenotypes induced by the pairwise interaction are more dependent on the presence of strains isolated from the same mycorrhizospheric-community or from distinct ones (sympatric or allopatric).
We isolated several *Streptomyces* strains from maize rhizospheres inoculated with different AMF species and tested their activity in monoculture and pair-wise combinations.
Our results indicated that changes in antimicrobial activity was linked to the strains of *Streptomyces* present in the interaction and its effectiveness varied with the indicator bacteria tested. We also found that interactions among sympatric microorganisms promoted the highest frequency of antagonistic phenotypes.
The results of this study suggest that the interactions between *Streptomyces* revealed specificities and mycorrhizal-induced local selection in antimicrobial activity. The growth of *Streptomyces* strains in mixed cultures which simulate biotic conditions that occur spontaneously in nature may be a useful approach in the induction of the production of new bioactive compounds.

Two widespread green *Neottia* species (Orchidaceae) show mycorrhizal preference for Sebacinales in various habitats and ontogenetic stages

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Plant dependence on fungal carbon (mycoheterotrophy) evolved repeatedly. In orchids, it is connected with a mycorrhizal shift from rhizoctonia to ectomycorrhizal fungi and a high natural ^{13}C and ^{15}N abundance. Some green relatives of mycoheterotrophic species show identical trends, but most of these remain unstudied, blurring our understanding of evolution to mycoheterotrophy. We analyzed mycorrhizal associations and ^{13}C and ^{15}N biomass content in two green species, *Neottia ovata* and *N. cordata* (tribe Neottieae), from a genus comprising green and non-green (mycoheterotrophic) species. Our study covered 41 European sites, including different meadow and forest habitats and orchid developmental stages. Fungal ITS barcoding and electron microscopy showed that both *Neottia* species associated mainly with non-ectomycorrhizal Sebacinales Clade B, a group of rhizoctonia symbionts of green orchids, regardless of the habitat or growth stage. Few additional rhizoctonias from Ceratobasidiaceae and Tulasnellaceae, and ectomycorrhizal fungi were detected. Isotope abundances did not detect carbon gain from the ectomycorrhizal fungi, suggesting a usual nutrition of rhizoctonia-associated green orchids. Considering associations of related partially or fully mycoheterotrophic species such as *Neottia camtschatea* or *N. nidus-avis* with ectomycorrhizal Sebacinales Clade A, we propose that the genus *Neottia* displays a mycorrhizal preference for Sebacinales, and that the association with non-ectomycorrhizal Sebacinales Clade B is likely ancestral. Such a change in preference for mycorrhizal associates differing in ecology within the same fungal taxon is rare among orchids. Moreover, the existence of rhizoctonia-associated *Neottia* spp. challenges the shift to ectomycorrhizal fungi as an ancestral pre-adaptation to mycoheterotrophy in the whole Neottieae

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Function by form - a tentative insight to the link between growth and the diversity of ectomycorrhizal fungi

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The means by which trees establish and utilize fungi is intriguing given the lack of host specificity shown by many fungal species. In order to reveal the mechanism by which fungal species richness affect forest productivity we studied the interaction of Norway spruce (*Picea abies*) seedlings and ectomycorrhizal (EM) fungi. We addressed how susceptibility to EM infection and the composition and functionality of the associated EM fungal communities relate to host genotype and their long-term growth performance. We found that Norway spruce genotype partly controlled the EM colonization of seedlings. However we found no relationship between the host future growth performance and the established EM communities or the functional capacity of single ectomycorrhizas; seedlings representing contrasting future phenotypes were equally colonized by EM fungi and the potential exoenzymes activities varied only according to the colonizing EM fungal species, not between host geno- or phenotypes. Nevertheless, the short root architecture, which was found to be a moderately heritable trait, varied consistently between seedlings of contrasting future phenotypes. One year old seedlings known to show fast growth in a later stage had sparse and widespread rootlets compared to seedlings representing the stunted future phenotypes. Norway spruce does not seem to show strong genetic signal for within-population selection towards its EM fungi at the species level. The superior growth of some spruce phenotypes may be a consequence of resource allocation and optimal root structuring in the juvenile stage rather than the extent of colonization by EM fungi. We accept that root physiological factors may subsequently lead to a higher capacity for symbiotic interactions in heterogeneous forest soil. An adequate and versatile means of nutrient acquisition is an important factor enabling fast growth, but might also provide the basis for positive feedback via enhanced mutualistic fungal interactions.

Comparative proteomics analysis of *Bacillus amyloliquefaciens* SQR9 revealed the key proteins involved in in situ root colonization

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Bacillus amyloliquefaciens SQR9 is a well-investigated plant growth-promoting rhizobacteria (PGPR) with strong root colonization capability. To identify the key proteins involved in in situ root colonization and biofilm formation, the proteomic profiles of planktonic and root colonized SQR9 cells were compared. A total of 755 proteins were identified, of which 78 and 95 proteins were significantly increased and deceased, respectively, when SQR9 was colonized on the root. The proteins that were closely affiliated with the root colonization belonged to the functional categories of biocontrol, detoxification, biofilm formation, cell motility and chemotaxis, transport and degradation of plant polysaccharides. A two-component system protein ResE, was increased 100 folds when compared with the planktonic status, impairment of the *resE* gene postponed the formation of cell biofilm and decreased the root colonization capability, which may be regulated through the *spo0A-sinI-yqxM* pathway. The SQR9 proteomic data provide valuable clues for screening key proteins in the plant-rhizobacteria interaction.

Fish emulsion as a food base for halophilic actinomycetes promoting growth of *Salicornia bigelovii* in a sandy soil in the United Arab Emirates

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Salicornia bigelovii is a promising halophyte found along the coastline of many countries including the United Arab Emirates (UAE). *S. bigelovii* is an environment friendly annual, leafless, succulent halophytic oil-bearing plant that can be readily grown on untreated seawater which is found along the coastline of many countries. Commercial fish emulsion was evaluated as a plant growth medium and as a nutrient base to enhance *S. bigelovii* growth by halophilic actinomycete inoculants. Five halophilic actinomycetes were selected from 42 actinomycetes based on their ability to produce plant growth regulators (PGRs), and to colonize *S. bigelovii* roots. These inoculants were tested in the presence and absence of autoclaved and non-autoclaved fish emulsion. The nutrient contents and types and levels of PGRs in tissues of treated plants were assayed to determine the basis of growth promotion. Fish emulsion was found to support plant growth in a sandy soil as effectively as an applied inorganic fertilizer. The plant growth promotion by the actinomycetes inoculants was most pronounced in the presence of autoclaved or non-autoclaved fish emulsion. The actinomycetes inoculants were capable of producing auxins, gibberellins and cytokinins and appeared to use fish emulsion as a source of nutrients and precursors for PGRs. PGR levels in planta following combined treatments of inoculants and fish emulsion were found to be significantly enhanced over other treatments. The value of fish emulsion appears to be more related to its role as a nutrient base for the inoculants rather than for the increased activity of the general soil microflora. These results also indicate that these treatments can be effective and economical for the cultivation of *S. bigelovii* in sandy soils such as those found in the UAE. The current study demonstrates for the first time the feasibility of using halophilic marine actinomycetes to promote the growth of *S. bigelovii* cultivated under greenhouse and potentially for field application for forage and seed production in proposed seawater irrigated production of *S. bigelovii*. This enhancement of growth by actinomycetes in the UAE is expected to increase the large scale production of *S. bigelovii* biomass that can be utilized not only for feed and culinary purposes but also for large scale production as a source of biofuels.

Effects of substrate complexity and temperature on growth of different microbial groups

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Soil microorganisms are one of the major contributors to terrestrial ecosystem carbon fluxes. Since soil microbial communities potentially mediate the feedbacks to climate change, understating their response to increasing temperatures is central if we want to predict the climate-induced changes in carbon fluxes in different ecosystems. Furthermore, under changing environments, e.g. temperature variation and availability of organic matter of varying complexity, it is still unclear in which way microbial communities will change their structure and/or functions. Here, we present results from a lab-based microcosm study where soil microbial communities were exposed to different temperatures and organic matter of different stability, simultaneously. Soil samples originated from vegetated and bare fallow subplots of two agricultural fields in southwest Germany varying in both climatic and edaphic conditions. Soils amended with cellobiose (CB), xylan or coniferyl alcohol (CA, lignin precursor) were incubated at 5, 15 and 25°C. Substrate decomposition was followed by measuring soil respiration during the incubation period. We generally found highest cumulative respiration (CO_2-C) at 25°C in all substrate treatments even though total microbial growth (measured as total extracted DNA) was higher at 15°C. Fungal biomass (measured as ergosterol content) showed significantly different response to added substrate and incubation temperature in both study areas, with higher ergosterol contents at 5 or 15°C compared to 25°C in all substrate treatments. Xylan addition resulted in significantly higher ergosterol contents than CB and CA. Within region, land use (bare fallow and vegetated subplots) also significantly affected the temperature response of fungal biomass to added substrate.

Furthermore, we are currently analyzing PLFA and qPCR (quantified using taxa specific primers) data in order to understand shifts in microbial community composition and the reaction of different bacterial taxa as a response to temperature and substrate complexity variations.

These results demonstrate the importance of interacting effects of soil temperature and availability of varying substrate quality in controlling microbial community functions and growth strategies.

Carbon-starvation in light induced tolerance to hyperosmotic stress in purple photosynthetic bacterium *Rhodopseudomonas palustris*

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Bacteria in soil frequently face to various stresses such as starvation, desiccation and osmotic pressures. It has been reported that tolerance to stresses in some bacterial species was increased under nutrient-starvation conditions. However, little is known about purple photosynthetic bacteria. We are studying on the effects of cellular energy on non-growing cells in purple photosynthetic bacteria. We have reported that a photosynthetic bacterium, *Rhodopseudomonas palustris* belonging to the order Rhizobiales, utilized light energy to enhance survivability under carbon-starvation conditions. In this study, we compared the tolerance to hyperosmotic stress of *R. palustris* cell starved in the light and dark.

R. palustris CGA009 was cultivated at 30°C in a carbon-limited medium (0.05% of Na-succinate) under illumination. Growth was terminated at exponential growth phase when the carbon source was depleted. The growth-terminated cells were further incubated anaerobically under starvation conditions in the light or dark. We compared stress tolerance among growing cells, growth-terminated cells, starved cells in the light, and starved cells in the dark. An osmotic stress was applied to the cells by incubation in a carbon-free medium supplemented with 2.5 M NaCl for 60 min in the dark. Viable cell number was determined by plate counting and survival rate under stress conditions was represented as a percent of CFU determined after 60-min incubation without NaCl.

The osmotic stress obviously reduced the survival rate of the growing and growth-terminated cells to 3-7%. The starved cells in dark also showed low stress tolerance. However, the 1-d starvation in light increased the survival rate to 40-100% and the high survivability was kept after 3-d starvation.

It is known that unsaturation degree of cellular fatty acids affects the membrane fluidity and tolerance to osmotic stresses. Composition ratio of the major cellular fatty acids (C16:0, C16:1, C18:0 and C18:1) did not vary among the cells tested in this study.

This study found that carbon-starvation increased the hyperosmotic tolerance in *R. palustris* and this starvation-induced adaptation to the stress occurred in the light. Our results suggest that cellular energy obtained through photosynthesis is utilized to make the starved cells adaptive to the osmotic stress without marked change in the cellular fatty acids composition.

Microbial communities inhabiting the rhizosphere of halophyton plants living nearby Hungarian soda lakes

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Many halophytes and halophilic microorganisms are capable to adapt to the extremities of saline habitats. This study was conducted to compare the genetic diversity, metabolic activity and ecological tolerance of rhizosphere bacterial communities of four halophyton plants living nearby Kiskunság soda ponds (Hungary). Denaturing Gradient Gel Electrophoresis (DGGE) was used to analyze genetic diversity. Metabolic activity of microbial communities was tested by basic (RESP) and substrate induced (SIR) respiration. Bacterial isolates were identified on the basis of 16S rRNA gene sequencing following the ARDRA grouping. Salt and pH tolerance of the isolates were examined by measuring their growth in broths containing 0-15% NaCl (w/V) and characterized with pH 7-12 values. On the basis of DGGE patterns, bacterial communities were clustered into two major groups according to geographical locations, physical and chemical properties of the rhizosphere samples. The results of SIR indicated that rhizosphere microbial communities had a high metabolic potential which could be stimulated by adding an easily metabolizing substrate. Among the isolates genera of *Anaerobacillus*, *Bacillus* and *Exiguobacterium* (Firmicutes), *Agromyces*, *Isoptericola*, *Microbacterium*, *Micrococcus*, *Nocardiopsis*, *Nesterenkonia* and *Streptomyces* (Actinobacteria), *Halomonas* and *Idiomarina* (Proteobacteria) and *Anditalea* (Bacteroidetes) were identified. The salt tolerance of the bacterial strains was strongly dependent on the sampling location and plant species. In contrast, growth of bacterial strains in broths with alkaline pH values was more balanced.
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Disturbance of the bacterial cell wall specifically interferes with biofilm formation

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In nature, *Bacillus subtilis* resides in multicellular communities, also called biofilms. In a biofilm cells are embedded in a self-produced extracellular matrix, a network of biopolymers composed of polysaccharides and proteins. In Gram-positive bacteria, the cell wall is the anchor point for the extracellular matrix. Much research is focusing on small molecules that manipulate and prevent biofilm assembly by modifying cellular signaling pathways. However, the bacterial cell wall, presenting the interface between bacterial cells and their surroundings is largely overlooked. We performed a systematic analysis of cell wall components that specifically disturb biofilm formation with minimal effect on planktonic growth. Biofilm development was dramatically disturbed by non-canonical D-amino acids, depletion of cell wall polysaccharides and specific interference with peptidoglycan assembly. Strikingly, none of these cell wall disturbances interfered with the transcription of major biofilm regulators and extracellular matrix genes. However, a careful analysis of the biofilm morphology revealed that these specific cell wall modifications altered the localization and anchoring of the extracellular matrix. In addition, we provide convincing evidences that biofilm hampering by peptidoglycan transglycosylation inhibitors and D-Leucine triggers a highly specific response in the protein profile within the biofilm cells, without changing the overall protein levels or the overall abundance of the extracellular matrix components. The diminished organization of the extracellular matrix through specific interferences with the cell wall highly sensitized biofilms to sterilizing agents. In conclusion, our results emphasize the central role of the Gram-positive cell wall in biofilm development, resistance and sustainment.

Alleviating the N limitation expands the possibilities for structuring soil bacterial communities: evidence based on the impacts of 5 years' manipulation of N dose and form in a Mediterranean ecosystem

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Enhanced nitrogen (N) availability is one of the main drivers of biodiversity loss and degradation of ecosystem functions. Mediterranean Basin ecosystems are nutrient-limited biodiversity hotspots, but no information is available on their medium- or long-term responses to enhanced N input. To understand the effects of increased N availability on a N-limited and highly biodiverse ecosystem, an N-manipulative experiment (N dose and form) was set up in 2007 focusing on the structural and functional changes in the interactions between the above and belowground communities..

The study site was in Serra da Arrábida (Portugal), soil (silt-sand-loam) is 15-20 cm deep, the vegetation consists of a dense maquis dominated by *Cistus ladanifer* (Cistaceae) and *Genista triacanthos* (Fabaceae) is also abundant. Plots of 400 m^2 (in triplicate) received no N addition (control), and treatments received 40A - 40 kg NH_4^+-N; 40AN - 40 kg NH_4NO_3-N; or 80AN - 80 kg NH_4NO_3-N ha^{-1} yr^{-1}. One soil sample (0-1 cm depth) was collected under the influence of 5 plants of *C. ladanifer* and *G. triacanthos* in each plot (October 2012). Soil samples were analyzed by large-scale pyrosequencing-based analysis of 16SrRNA gene sequence.

After filtering low quality sequences, OTU clustering and singleton and chimeric sequences removal, the number of reads was 116,007 and of total OTUs was 6,045. Sixteen bacterial Phyla were represented, from which only four (Proteobacteria, Actinobacteria, Acidobacteria and Gemmatinmodates) represented 95% of the data.

The NMDS ordination revealed that a predictable bacterial community was associated with the control soil samples. While alleviating the N limitation (N treatments) 'removed the constraints' resulting in a more stochastic community. Analyses of the indicator species showed that control plots had higher number of indicator species (58) followed by the 40AN, 80AN and the 40A plots. There were 8 bacterial phyla represented within the indicator species. Proteobacteria were indicator for the 4 treatments, however, Acidobacteria were only for the Control and 40AN plots. Whereas, Actinobacteria were indicator for the Control and the 80AN plots.

The results clearly show that under stress conditions the soil bacterial community was well structured, indicating that bacteria were recruited based on their specific functionality, while alleviating stress conditions lead to a community more influenced by the stochastic events.

Soil microbial and nematode communities respond differently to warming and plant functional group removal across a post-fire boreal forest successional gradient

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Global warming is causing increases in surface temperatures and has the potential to influence the structure of soil microbial and faunal communities. However, little is known about how warming interacts with other ecosystem drivers, such as vegetation composition or successional stage, to affect the soil community and thereby alter ecosystem functioning. We investigated how experimental warming, moss removal and shrub removal along a post-fire boreal forest successional gradient impacted on soil microbial and nematode communities. Our results showed that warming altered soil microbial communities and favored bacterial-based microbial communities, but these effects were mediated by mosses and shrubs, and often varied with stand age. Meanwhile, the nematode community was generally unaffected by warming and positively affected by the presence of mosses and shrubs, with these effects mostly independent of stand age. These results highlight that different groups of soil organisms may respond dissimilarly to interactions between warming and the understory vegetation, with likely consequences for ecosystem functioning that may vary with stand age. Due to the ubiquitous presence of shrubs and mosses in boreal forests, the effects observed in this study are likely to be significant over a large proportion of the terrestrial land surface. Our results demonstrate that it is crucial to consider interactive effects between warming, understory vegetation and successional stage when predicting soil community responses to global climate change in forested ecosystems.

Impacts of N enrichment on Mediterranean biological soil crusts community and functions: the unseen evidence from soil pigments

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Without the use of nitrogen fertilizers, the human population would be approximately half of what it is but N pollution affects air, water, soil, climate and ecosystems, which in Europe costs 70-320 billion € per year. Although the Mediterranean Basin is a biodiversity hotspot threatened by N enrichment, very few addressed the impacts on belowground. Biological soil crusts include cyanobacteria, algae, fungi, bryophytes and lichens, found at the soil surface play an important role in the N cycle namely by fixing N. Therefore, we focused on the impacts of N enrichment on soil pigments, as indicators of shifts in soil microbial communities, and related them with N fixation. For that we took advantage of an N-manipulation field experiment that has been running since 2007. The study site is in Serra da Arrábida (Portugal) and estimated ambient N deposition is < 4 kg N ha^{-1} yr^{-1}. The soil is 15-20 cm deep and has a silt-sand-loam texture. The vegetation consists of a dense maquis dominated by *Cistus ladanifer* L. (Cistaceae) and *Genista triacanthos* Brot. (Fabaceae) is also abundant. Control plots received no N addition, while there are three N treatments: 40A received 40 kg NH_4^+-N ha^{-1} yr^{-1}; 40AN received 40 kg NH_4NO_3-N ha^{-1} yr^{-1}; and 80AN received 80 kg NH_4NO_3-N ha^{-1} yr^{-1}. Each treatment has three replicates (400 m^2 experimental plots).
One soil sample (0-1 cm depth) was collected under the influence of 5 plants of *C. ladanifer* and *G. triacanthos* in all experimental plots in October 2012. Soil nitrogenase activity was determined by acetylene reduction and soil pigments using HPLC analysis on acetone-extracted samples.
Under natural conditions (i.e., no N additions), and irrespective of the plant species, sytonemin (exclusive to cyanobacteria) was the most abundant soil pigment suggesting that cyanobacteria were dominant. However, adding N shifted the soil communities into becoming dominated by green algae (significant increase in chlorophyll b, violaxanthin and lutein) and other microorganisms (significant increase in chlorophyll a). In agreement, adding N, especially 40A, impacted negatively soil N fixation but only in the samples under the influence of *G. triacanthos*. Soil pigments may be good indicators of the impact of N enrichment in Mediterranean maquis as they are very sensitive and integrate ecosystem functions.

Unveiling the blueprint of marine-terrestrial transition in bacterial adaptation and evolution

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The marine-terrestrial transition represents one of the most fundamental and yet overlooked theme in microbial adaptation and evolution. This is in part caused by the lack of a suitable environmental gradient that allows the investigation of community dynamics across a comprehensive time-scale. Here we investigated the ecophysiological adaptations of bacterial communities using shotgun metagenomic analysis across a natural salt marsh chronosequence spanning over a century of ecosystem development. We predict communities on the marine or terrestrial extremes to follow multifaceted ecophysiological patterns, herein called 'flight or fight mode'. In brief, communities thriving in more aquatic environments would be potentially more adapted to sense and move along chemical gradients ('flight' mode); whereas communities adapted to terrestrial conditions were expect to be less motile and highly aggregated, leading to an increase in competitiveness – here represented by genes associated with antibiosis and cell persistence ('fight' mode). First, comparative metagenomics revealed that genes involved in bacterial chemotaxis and flagellar-motility – pathways that confer the motile chemosensory behavior to cells – were overrepresented at initial soil sites, sharply decreasing at latest successional stages. Noteworthy, these initial sites are boundary soils regularly subjected to overflow by seawater, meaning that microbial cells holding ecological traits that allow for an effectively perception and exploitation of microscale patches in a diffusible environment may have selective advantages. In contrast, as soils mature in our system, the gradual decrease in inundation frequency leads towards a less diffusible habitat, in which the best strategy is to 'fight your enemies'. Indeed, we found genes involved in antibiotic biosynthesis (e.g. polymyxin, phenazine) and antibiotic resistance (e.g. beta-lactamases), as well genes involved in cell persistence (*hipAB* module) and carbon uptake to be overrepresented at latest successional stages. Thus indicating that chemically-mediated mechanisms associated with biotic interactions may potentially provide fitness gain for terrestrial populations. Together, our data provides evidence that, particularly in terrestrial environments, the multitude of microhabitats and nutritional limitation are expect to exert a selective pressure in organism interactions to effectively establish and compete for local resources.

Bacterial antibiotic resistance heterogeneity in natural subterranean habitats

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The overuse of agents with antibiotic effects in last 70 years resulted in a global health problem associated with reducing of a therapeutic potential of antibiotic drugs. A comprehensive and global strategy to combat a spread of antibiotic resistance (ATB-r) is necessary. A better understanding of the relationship between the environment and the ATB-r of bacteria is important for indication of ATB-r reservoirs and risk of the ATB-r dissemination.
We studied heterogeneity and potential of ATB-resistance of cultivable bacterial communities in different natural habitats (soil and cave sediments) from 11 protected cave localities (Slovak Republic, Central Europe). In total, 350 ATB-r dominant bacterial cultures (isolated on tryptic-soy agar) were screened for resistance to 15 antibiotics and identified to species (analyses of 16S rRNA gene sequences). They were grouped by habitat type of their isolation (soil, bat guano, mineral sediments of the entrance, center and terminal part of the caves) and used together with substrate characteristics for multivariate analyses and models (Canoco5) for description and comparison the ATB-r patterns of bacterial communities in soil and cave environment and for identification of environmental factors affecting the ATB-resistance. Results showed that the ATB-r patterns differed among habitat types ($p=0.047$) as well as among localities ($p=0.001$). The incidence of ATB-resistance was higher in cave with the active underground rivers than in dry caves. The representatives of Proteobacteria and Bacteroidetes phylla were confirmed as taxons with higher frequency of ATB-multiresistance in comparison to the other taxons. Bat guano was identified as risky reservoir of ATB-r pathogens. This contribution extends our knowledge of the occurrence of ATB-r in subterranean environment, and shows that natural microbiomes and environments, which are not affected directly by practice of antibiotic application, should not be neglected as important reservoirs of ATB-resistance.

Influences of Long-term Nitrogen Fertilization on Fungal Endophyte Community of Three Grasses in an Alpine Meadow

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A 4-year nitrogen fertilizer(NH_4NO_3) gradient was applied to plots in an alpine meadow ecosystem to address how grass fungal endophytes (FEs) respond to long-term fertilization.High-throughput sequencing of foliar FEs of three grasses revealed that 1) Over 95% of FEs in surface-sterilized leaves belonged to Fungi Unclassified, while the number of the non-surface-sterilized was less than 0.07%, showing that FEs research has only just begun. 2) FEs of family Lasiosphaeriaceae significantly reduced under high nitrogen fertilizer inputs (10 g m^{-2} yr^{-1} and/or 15 g m^{-2} yr^{-1}) compared to control (0 g m^{-2} yr^{-1}) and/or low fertilizer inputs (5 g m^{-2} yr^{-1}) in all three species. Similarly, FEs of family Davidiellaceae and Dothioraceae, Sporormiaceae and Nectriaceae remarkably decreased under high nitrogen fertilizer inputs in *Anemone rivularis* var. flore-minoreand Elymusnutansleaves, respectively, and the same tendency was discovered in Filobasidiaceaeendophyte in Thermopsislanceolata. However, member of family Togniniaceae and Entylomataceae in Elymusnutans increased under high nitrogen fertilizer inputs. 3) Diversity and richness of FE community varied among three species, and richness of FE community of Thermopsislanceolatacorrelated with both plant C, N, P concentration and nitrogen fertilizer, which revealed that fertilization had an impact on plant FEs. Additionally, some parasitic endophytes were discovered to show pronounced dependency or contingency on environmental conditions. Indicating that the effect of FEs on their host is not predominantly benefit as early researchers maintained, but is variable and dependent on the host and the environmental circumstance that they are found.

The transport of marine phages in soil as a tool of understanding the interaction of surface-subsurface events

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Little is known about the factors determining the eligibility of marine phages as specific markers of hydrological flow and reactive transport of colloidal particles in the Earth's Critical Zone (CZ). Marine phages and their bacterial hosts are naturally absent in soil, can be applied as tracers at high concentration and be detected as little as one phage/mL in water. Within the DFG Collaborative Research Center – AquaDiva we evaluated the effects of phage characteristics (e.g. morphology, surface property), on transport of marine phages in laboratory experiments. Phages were characterized by electron microscopy, dynamic light scattering and water contact angle analysis (CA) for their size/morphology, surface charge (?) and hydrophobicity. Sand-filled percolation columns and a modified high-throughput plaque assay were used to quantify phage deposition during percolation.
Our data show that all marine tested phages exhibited relatively low deposition efficiencies and high transport rates in our laboratory setups. Transport rates depended mainly on the size, hydrophobicity and potentially on morphology of the phages tested. Despite of similar morphology (Siphoviridae) and surface charge (-13 mV) of the two phages PSA-HS2 and H40/1, the bigger (60 nm) and less hydrophobic (CA = 40°) PSA-HS2 phage exhibited a lower deposition efficiency than the smaller (39 nm) and more hydrophobic (CA = 52°) H40/1 phage. On the other hand, HM1 phage exhibited higher deposition efficiency than PSA-HS2, although both have similar characteristics with one exception whereas HM1 has different morphology (Myoviridae). We conclude that marine phages have a high potential for the use as sensitive tracers in terrestrial systems. The use of phages as markers contributes to a better, mechanistic understanding of the drivers of colloidal transport in subsurface ecosystems.

Hydration dynamics in desert soil mediate antagonism of actinobacteria

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Assemblage of organisms in communities were often linked to antagonistic interactions mediated by chemical warfare through secondary metabolites. Desert soil is especially rich in diverse communities of actinobacteria, known for their capacity to synthesise secondary metabolites. We have observed that during rainfall the abundance and diversity of actinobacteria are significantly reduced, yet as the soil desiccate they quickly regain their dominance. We did not understand how these fluctuations in diversity are ecologically maintained in a dry environment seldom interspersed by rainfall but we speculate that antagonistic interactions are a shaping force in the community assembly. We, therefore, analysed antagonism among actinobacteria isolated from desert soil during rainfall and drying event. We hypothesized that the frequency of antagonism among the actinobacteria isolated when soil is hydrated and competition between species is fierce would be higher than during desiccation when the communities are segregated. To test our predictions, 53 actinobacteria were randomly isolated at different time points during hydration-desiccation and were shown to share limited taxonomic relatedness. Almost 3000 antagonistic interactions were performed and then evaluated by geostatistic models, creating efficient hydration networks with hierarchical structure. Sensitive, resistant and antagonistic isolates co-occurred at each time point, but during extreme desiccation and total hydration antagonistic interactions were more pronounced than when the soil was wet and drying. The distribution of the interactions was not random but could be explained by reciprocity, as strain A inhibited the growth or excluded strain B it was most likely that strain B will reciprocate. Such reciprocity was enhanced in strains derived from the same time point, suggesting that it may be the property of a community responding to soil hydration. This is the first report on the prevalence and specificity of actinobacteria interactions following rainfall in the desert and provides evidence for the influence of hydration-mediated assemblages on soil bacterial antagonist interactions.

Ectomycorrhizal fungal spore bank recovery after a severe forest fire: Some like it hot

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Fires in western North America have transitioned from frequent, low intensity events to infrequent stand-replacing events. After severe wildfires, pine recovery depends on ectomycorrhizal (ECM) fungal spores surviving and serving as partners for regenerating forest trees. We took advantage of a large, severe natural forest fire that burned our long-term study plots to test the response of ECM fungi to fire. We sampled the ECM spore bank using pine seedling bioassays and high throughput sequencing before and after the California Rim Fire, which killed most or every tree in both plots. We found that ECM spore bank fungi survived the fire and dominated the colonization of in situ and bioassay seedlings, but there were specific fire fungi such as *Rhizopogon olivaceotinctus* that increased in abundance after the fire. The frequency of ECM fungal species colonizing pre-fire bioassay seedlings, post-fire bioassay seedlings, and in situ seedlings were strongly positively correlated. While the most abundant taxa remained the same, fire reduced the ECM spore bank richness and density: fewer species were detected in the spore bank after the fire and fewer of the samples had colonized seedlings. Our results show that although there is a reduction in ECM inoculum, the ECM spore bank community largely remains intact, even after a severe “mega-fire.” Furthermore, simple greenhouse bioassays can be used to determine which fungi will colonize after fires. Similar to plant seed banks, a specific suite of ruderal, spore bank fungi take advantage of open niche space after fires.

Effect of increased N availability on ammonium oxidizing bacteria populations – A possible bioindicator in Mediterranean ecosystem

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Increasing N deposition is one of the main global change drivers threatening the stability and functioning of Mediterranean ecosystems, a biodiversity hotspot with distinct conditions and a biological community adapted to them. Most impacts of increased N deposition, and consequently, increased N availability relate to changes in N forms and bio-transformations. We focused on a highly responsive, but not well studied process in these ecosystem: Nitrification.
We aimed to study the impact of increased N availability on ammonia oxidizing bacteria (AOB) and evaluate their use as bioindicator.
The study was conducted in a semi-natural ecosystem in Arrábida Natural Park where N availability has been modified by the addition of 0, 40 and 80 kg N ha^{-1} yr^{-1} in form of ammonia and/or ammonia-nitrate along 7 years. Soil from an agricultural system was used as positive control for nitrification. Soil proprieties, soil nitrification potential, activity and structure of cultured AOB populations were determined.
A low nitrification potential was observed for the semi-natural soils, with and without N addition. However, the AOB populations from soils with N-addition did had altered activity and structure. From the ammonia-nitrate treatment, AOB had potential activities similar to those found in agricultural soils, and for the ammonia treatment, AOB potential activity was even higher. For these semi-natural soils, the observed low soil nitrification potential and high potential AOB activity may indicate the existence of nitrification-inhibition mechanisms. Nonetheless, by culturing the AOB, structural and activity changes were observed. And as such, these may provide a detailed bioindicator of increased N availability.

Phylogenetic estimation of ecologically important traits illuminates microbial community responses to change in natural and agro-ecosystems

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Many types of environmental change - from global-scale changes in climate to field-scale changes in management - alter the composition of microbial communities. However, determining whether similar "types" of microbes are selected by similar changes, or by the same change across locations, remains difficult, limiting synthesis across studies. By allowing communities to be described in terms of the ecological types of taxa they contain, estimating trait values using 16S rRNA sequence-based phylogenetic placements could provide a way forward. We developed a reference tree of 1475 high-quality microbial genomes and used it to estimate values of three ecologically important traits: rRNA gene copy number (indicator of maximum growth rate), genome size (indicator of metabolic versatility) and anaerobic tolerance. Traits were estimated for soil microbial communities from two experiments: (1) a grassland experiment in which supplemental late-season precipitation and NPK fertilizer were added to adjacent high- and low-productivity soils; and (2) a long-term agricultural experiment comparing three management systems: organic (C+N inputs from cover crops and composted manure), conventional (N inputs from mineral fertilizer) and no input. Weighted average trait values for each community were calculated using OTU relative abundances. In the grassland, addition of nutrients resulted in higher copy numbers, larger genomes, and higher anaerobic tolerance, potentially selecting for faster-growing microbes with higher metabolic versatility. Supplemental precipitation produced the opposite set of responses. In the agricultural experiment, the organic system had higher copy numbers, smaller genomes, and higher anaerobic tolerance than the no input system, with the conventional system falling in the middle. Notably, although inputs to both the grassland and agricultural system selected for high copy numbers (potentially faster growth), the grassland nutrient addition increased average genome size (potentially higher metabolic versatility) whereas the agricultural carbon + nutrient inputs decreased it (potentially lower metabolic versatility). This difference may result from reduced soil habitat complexity or lower carbon substrate diversity in the agricultural system. In sum, community-wide estimation of microbial traits holds promise for ecologically meaningful prediction of environmental change responses, facilitating cross-system insight by providing a common currency.

Methodological Improvements to Amplicon-Based Surveys of Microbial Community Structure

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Deep amplicon sequencing of microbial ribosomal RNA genes is now routinely performed on large sample sets, due to the low cost of high-throughput next-generation sequencing. Such sequencing efforts are still dependent on polymerase chain reaction (PCR) amplification. The PCR is sensitive to mismatches between primer and template, and mismatches can lead to inefficient amplification of targeted regions of DNA template. In PCRs in which a degenerate primer pool is employed, each primer can behave differently. Therefore, inefficiencies due to different primer melting temperatures within a degenerate primer pool, in addition to mismatches between primer binding sites and primers, can lead to a distortion of the true relative abundance of targets in the original DNA pool. To help reduce the negative effects of PCR distortion, we developed a conceptual model leading to a novel amplification strategy, entitled “Polymerase-exonuclease-PCR (PEX)”, in which primer-template interactions and primer-amplicon interactions are separated. The PEX PCR method substantially and significantly improved the evenness of recovery of sequences from a mock community of known composition, and allowed for amplification of templates with additional introduced mismatches near the 3’ end of the primer annealing sites. We demonstrate that the method can be used to identify which primers interact with genomic DNA templates, and this empirical data may help improve the design of degenerate primer pools and allow for broader amplification of intended target sequences. We further demonstrate the efficacy of this method in a range of environmental samples, including soils and sediments, targeting both bacterial and fungal populations. This method is easy to perform, and is recommended when degenerate primer pools are used or when mismatches between primers and template are possible.

Fungi on mountainsides: contrasting elevational and seasonal patterns among root-associated fungal groups.

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Arbuscular mycorrhizal fungi (AMF) and dark-septate endophytes (DSE) are common alpine plant root-associated fungi, which often co-colonize root space, but have different functions. Both fungi increase plant nutrient acquisition, but DSE are more stress resistant. At our sites in China and the USA, both nutrient availability and temperature decline with increasing elevation and water availability decreases through the season, thus, we predicted that DSE would be more common on all plants at high elevations (nutrient and temperature stress) and increase their abundance across the gradient as the season progressed (drought stress). Across both gradients and across plant species, DSE colonization was higher at high elevations and AMF colonization was higher at low elevations. AMF were more abundant than DSE in roots early in the season. However, this pattern changed as the season progressed with AMF colonization declining and DSE colonization increasing (AMF $t = 2.3$ $p = .03$, DSE $t = 4.3$ $p<0.01$). Overall, shifts in fungal colonization along the gradient were stronger than the shifts across the growing season. Our results suggest that plant stress, due to water or nutrient availability, may drive similar shifts in root-associated fungal community composition across different mountains.

Spatial and seasonal variability of the microbial community in forest fen soils on North-East-Germany

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Fluctuating water levels in peat soils lead to spatial and temporal variations in soil respiration. How do these dynamics affect the microbial community structure?
We investigated five forest fen sites, characterized by different water regimes, in regard to their microbial community using PLFA-analysis technique.
All sites are dominated by gram-positive bacteria. However, we observed spatial differences in the microbial biomass in relation to the water table. Sites with a higher water level exhibit larger amounts in comparison to sites with a lower level (2-3 times higher).
Seasonal variation in the microbial composition seems to be negligible.

The Contingency of some Biotic and Abiotic Parameters in Arable Land and Permanent Grasslands

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The contingency of biotic and abiotic parameters of two different study sites (Tajov - TA in Kremnica Mountain, Liptovská Teplička -LT in Low Tatras Mountain) with two different land use (arable land and permanent grasslands) were analysed. Biotic (microbial community structure, earthworm number and earthworm fresh body biomass) and abiotic chemical soil parameters (pH in KCl, soil organic carbon, total nitrogen, nutrients) were measured.
According to MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) analyse several bacterial strains were identified in arable land, like *Bacillus cereus*, *Pseudomonas chlororaphis*, also *Sporosarcina psychrophila*, with interesting ecological atribute like urea utilisation ability. Permanent grasslands microbial composition showed frequently *Bacillus cereus*, *Bacillus thuringiensis*, *Serratia fonticola*. These findings, added also by dendrograms, are corresponding with prevalent autumn and spring maximums of microbial activity and diversity in arable land, because of plowed agricutural works and amylolytic, proteolytic abilities of microrganisms. For observation of changes in soil microbial communities the Biolog plates method was used.
At TA and LT the average density of earthworms per one m^2 of arable land was 31.5 individuals.m^{-2} and 33.8 individuals.m^{-2} respectively. At TA and LT the average density of earthworms per one m^2 of permanent grasslands was 95.6 individuals.m^{-2} and 87.5 individuals.m^{-2} respectively. Higher body biomass and density of earthworm individuals in grasslands then in arable land showed that land use connected with specialised management practices directly influence the spatial distribution as well as functioning ecology of earthworms in lands.
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Key words: soil microorganisms, MALDI, arable land, permanent grassland

Characterization of microbial pool in sub-kurgan palaeosols of different ages in desert-steppe zone in relation to the holocene dynamics of climate

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Ground burial mounds (kurgans) in Russian steppe zone preserve palaeosols, which are good objects for reconstructing the dynamics of Holocene climate. The work presents comparative characterization of microbial pool of palaeosols buried beneath kurgans dated back to Eneolith, Bronze, and Middle Age time-windows (4200 ?? – AD 1300) and their surface analogs.
From physicochemical properties of the soils, the dynamics of climate in the second half of the Holocene was reconstructed. Within the Eneolithic (4200-4100 BC) and Middle Age (AD 1300), time-windows the local climate was more humid than the current one. In III mil. BC, the climate aridity increased with maximum timed to its end. In surface soil profiles, total microbial biomass varied from 225 to 530 μg C/g and comprised 4-16% of soil Corg in A1 horizon and up to 76% in deeper ones. In A1 horizon, the microbial biomass was almost completely alive and active. Down the profiles, the share of alive and active microbial biomass decreased respectively to 20 and 10% of the content in A1 horizon. In buried palaeosols, the state of microbial pool depended on palaeoecological conditions of the time of burial. Total microbial biomass exceeded current level by 50% in the Eneolithic, 2 times – in the Bronze time palaeosols, and was comparable to it in palaeosol of the Middle Ages. Total microbial biomass comprised 6-26% of the Corg preserved in the palaeosols. The share of alive biomass in microbial pool varied from 11 to 100% and was highest in palaoesol of the Bronze time-window (2200-2100 BC). The share of microorganisms utilized plant residues increased in palaeosols of the Bronze time. The share of active microbial cells was 1-56% of alive biomass and was maximal in the Middle Age palaeosol. Both in surface soil and palaeosols studied the number of microorganisms averaged to 49 x 109 cells/g. Concluding, in palaeosols microorganisms do not lose viability under dry conditions and long-term deficiency of nutrients. The sizes of microbial pools are comparable or exceed those of surface soil. The ecological trophic structure and activity of microbial communities are connected to the level of climate humidity in the region studied.
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Hot spots and cold spots – modelling biodegradation dynamics under disturbance regimes

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Microbial degradation of soil pollutants is an important ecosystem function. As soil systems are constantly exposed to disturbances of different spatial appearences and frequencies, their ability to recover the biodegradation function is crucial. However, the effect of disturbance regimes to the long-time biodegradation dynamic is not yet been examined. We applied a numerical simulation model considering growth, degradation and bacterial dispersal to analyze the spatiotemporal dynamics of biodegradation in response to disturbance regimes. To investigate the influence of bacterial distribution, we simulated different spatial configurations of the disturbances with various degrees of fragmentation. Moreover, we considered bacterial dispersal networks to simulate bacterial movement along fungal networks in soil.We found that the biodegradation performance decreases in response to periodic disturbances but tends to saturate around a mean. Spatiotemporal observation of degradation dynamics reveal partitioning of the system in active hot spots and inactive cold spots. In hot spots the function is maintained on a certain level of saturation, whereas in cold spots degradation is completely down. However, size and distribution of hot/cold spots depend on spatial configuration of the disturbance. We identify a critical degree of fragmentation of the disturbance under which the degradation activity concentrates on hot spots. Is the disturbance higher fragmented the whole system is homogeneously active. This effect is due to variation in mean distance between disturbed and undisturbed area. The more distant a disturbed habitat from the next undisturbed the longer the lag-phase of biodegradation recovery and thus the system could absorb higher fragmented disturbances better. However, if dispersal networks are applied this critical degree is shifted as the networks are increasing bacterial dispersal. Our results show that ecological interaction in reaction to the disturbance pattern is responsible for maintaining the biodegradation performance under disturbance regimes. The degree of fragmentation of the disturbance configuration influences the dynamics which indicates the relevance of spatial processes for functional stability. Besides, fungal networks may increase activity by enhancing bacterial dispersal. However, the influence of fungal networks to the degradation dynamics also regarding other functionalities like substrate/water transport should be analyzed further.

Characterization of mycorrhizophere in a Hungarian saline-sodic grassland

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Arbuscular mycorrhial fungi (AMF) are beneficial endosymbionts, that can improve the water and nutrient uptake of hosts and help plants to get over different environmental stresses. Therefore, AMF have an important effect on the structure and the diversity of plant communities.
For conservation treatments and to preserve the natural environment in the Hungarian steppe temporal and spatial changes of the plant communities and the influencing factors behind this were examined. Our knowledge on AM fungi community in saline soils and their role in drought and salt stress is very limited.
Four typical plant communities, such as *Lepidio crassifolii-Puccinellietum limosae* ("mézpázsitos"), *Artemisio santonici-Festucetum pseudovinae* ("ürmöspuszta"), L*epidio crassifolii-Camphorosma annuae* ("vakszik") and *Achilleo-Festucetum pseudovinae* ("sziki legelő") were examined in saline-sodic soils of Apajpuszta (Kiskunsági National Park; Hungary).
The salt concentration and the pH of the soils showed a descending and the humus content showed a growing gradient in the order of "vakszik"-"mézpázsitos"-"ürmöspuszta"-"sziki legelő".
The mycorrhizal status of the most abundant plants and the diversity of AMF communities were examined with PCR-RFLP and DGGE methods. In "vakszik" plant community mostly non-mycotroph plants were found. In "ürmöspuszta" and "sziki legelő" communities mycotroph plants were in a higher ratio and in "mézpázsitos" the mycotroph and less mycorrhiza dependent *Puccinellia limosa* could be found in more than 90 % abundance.
Similar tendencies of microbial functionality were shown by the fluorescein diacetate test, the soil catabolic activity pattern and the measurement of glomalin-related soil proteins. The "vakszik" community always had the lowest values; while the other three variously differed significantly from each other.
Density of AMF infective propagules were estimated by the amount of mycorrhizal root colonization in most probable number (MPN) test. We observed remarkable differences between the AMF infectivity in the soils. The "vakszik" and "mézpázsitos" plants had very low root colonization and no colonization in the MPN test. The "mézpázsitos" soil was flooded which blocked AMF infection. In "ürmöspuszta" and "sziki legelő" 40-70 % (M% Trouvelot et al. 1986) colonization intensities were found and soils contained low density of infective propagules in the MPN test.
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The first study of actinomycetes complexes in Prietonie region soil

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Lake Elton is a highly saline lake in Volgograd province, Russia, near the Russian border with Kazakhstan. The lake occupies an area of 152 km2 and is only 0.3–0.6 m deep. It is 18 m below sea level. Elton is considered to be the largest salt lake in Europe. The region around the lake is called Prieltonie.
Soils of Prieltonye are typical solonchak, light-brown soils and salt-dome structures.
Up to the present moment there there has been no information published or studies into the microbial communities of Prieltonie's soil. This work is a first research into actinomycete complexes of Prieltonie soils. In our study we have chosen soil catena situated along the shore of the Khara river that run into the lake and have taken 5 samples from different types of soils. Samples X1-X2 are typical solonchak soils; samples X3-X5 are light-brown soils under the saltwort.
The method of inoculation from dilutions of soil suspension on solid nutrient media has shown that the population of actinomycetes in typical solonchak soil is comprised of $2x10^2$ and $3 x10^2$ CFU/g of soil for samples X1 and X2 respectively, and comprised 100% and 7% of the total number of bacteria respectively. The amount of actinomycetes in light-brown soils (sample X3-X5) is greater is greater and amounts to 104 CFU/g of soil. Their number corresponded to 3% and 35% of the total number of bacteria in these three samples.
The high-throughput pyrosequencing method has shown a diversity of actinomycete varieties and the presence of such families as Nocardiaceae, Micromonosporaceae, Streptosporangiaceae, Streptomycetaceae, Frankiaceae, Promicromonosporaceae, besides such genes as *Streptomyces*, *Streptosporangium*, *Actinoplanes* and *Dactylosporangium*.
The highest level of actinomycetes diversity was found in the light-brown soil (sample X4) taken from a saltwort slope. This is probably because of the active desalination process of the soil on this land, and it's the heavy root system.
The presents of "rare" species of actinomycetes make Priletonie soils a prospect for biotechnological isolation of significant strains of mycelial actinobacteria. It's also interesting to unravel the evolution of actinomycetes and bacterial communities in such prototypes of ancient geological ecosystems as Prieltonie.
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Soil bacterial community along a successional series of tidal flats in the Yellow River Delta

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Tidal flats are critical components of coastal ecosystems and are characterized by high primary productivity and diversity. The bacteria in tidal flat sediments drive various biogeochemical processes. However, the structure and function of bacterial community in tidal flats are poorly understood. Successional tidal flat ecosystems form natural environmental gradients, which may influence bacterial community. Microbial communities also face large seasonal variations in temperature in temperate coastal regions. This study aimed to reveal how bacterial communities respond to the seasonal variation along a successional series of tidal flats (subtidal, intertidal and supratidal flats). Bacterial community composition and diversity from tidal flats were analyzed over four seasons by 16S rRNA genes using the Ion Torrent PGM platform. The relative abundances of Acidobacteria, Gemmatimonadetes, and Nitrospirae increased, while Chloroflexi, Actinobacteria, and Firmicutes decreased from subtidal to supratidal flat. Bacterial phylogenetic diversity increased, and phylogenetic turnover decreased, from subtidal to supratidal flat. Moreover, the bacterial community structure differed significantly among seasons. Temperature, Na^+, SO_4^{2-}, and nitrite and clay contents were the main drivers and explained 14.4% of the variability. Despite major compositional shifts, functional capacity predicted with PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) revealed high similarity in most of level-3 KEGG (Kyoto Encyclopedia of Genes and Genomes) Orthology groups. The predicted soil bacterial metagenomes in winter had lower relative abundances of functional genes associated with biogeochemical processes, such as nitrogen metabolism, methane metabolism, vitamins metabolism and energy metabolism. Taken together, our study indicates that the bacterial community structure and function in tidal flats shifted along the tidal flat gradient. This research provides new insights on the impact of a tidal gradient on bacterial community in coastal ecosystems.

Ecological determinants of soil bacterial community structure across multiple scales in a Mongolian global change experiment

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While a new generation of 'omics methods has rapidly advanced our understanding of microbial diversity and its drivers, much remains to be explored. When considering microbes in changing environments, it is important to evaluate potential responses to multiple stressors, while simultaneously accounting for existing community variation. We conducted a multi-year experiment to test the effect of warming and grazing on soil bacterial communities along a spatial gradient Mongolia where air temperatures have increased by 1.6°C since 1960 and traditional nomadic pastoralism is becoming more sedentary. We employed open-top chambers to passively manipulate climate by creating warmer and drier conditions, as well as year-round fencing to reduce grazing pressure in some plots. Soil samples were collected from 2010 - 2012 at upper and lower slope locations. Bacterial community structure was assessed by sequencing the 16S rDNA gene V4 region. Plant available N, soil temperature and moisture were measured from June - August. In 2012, point-source data included a larger range of edaphic variables. For 2010 - 2011 data, constrained analysis of proximities (CAP) examined effects of year, slope, climate and grazing on bacterial community structure using both Bray-Curtis (BC) and UniFrac (UF) analyses. Structure varied more between years than with slope, grazing or climate. The strong effect of year holds for both analyses, with slope significant in BC and climate in UF; this suggests that while the difference between slopes is only taxon-based, differences due to climate are phylogenetic, implying potential functional differences. Plot-level N, moisture and temperature gradients were significant using BC; the latter was not using UF. Preliminary 2012 data, assessed with UF, survey the effects of edaphic variables (ADONIS) and experimental factors (ANOSIM) on community structure. Overall, the community-wide response to experimental manipulation is more pronounced on the lower vs. upper slope, suggesting that historical communities on the lower slope may be comparatively less buffered to environmental change; which are likely subject to narrower ranges of edaphic fluctuations within and between years.

Soil fungal responses to warming in polar regions

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Temperature increases in polar regions are already having negative effects on ecosystems, but still little is known of how warming might affect polar soil fungi. Filamentous fungi are abundant in Arctic regions and are key components of Arctic soils. They are pivotal to many ecosystem processes, notably nutrient cycling. It is not known how climate warming will affect fungal community composition and activity, or nutrient cycling and soil organic carbon storage in cold environments.
A soil warming experiment was set up in autumn 2014 at Kvadehuken on the Broggerhalvoya Peninsula, Svalbard to address the effect of experimental warming on soil fungi. Open top chambers are used to warm soil, along with the addition of water to mimic changes likely to occur in the ecosystem in the future. Soil samples were gathered at time zero of the experiment, prior to application of any treatment, and will continue to be gathered on a yearly basis to follow soil fungal community changes over time.
Both molecular and conventional microbiological techniques are used in this study to analyse the fungal community and its response to warming. Fungi are identified by the PCR amplification of genes encoding the ITS region and by genetic fingerprinting using denaturing gel electrophoresis (DGGE). Microbiological techniques are used for soil fungi isolation and culturing to enable investigations of how growth rates, activity and extracellular enzyme production of different fungi present in Arctic soils are affected by altered temperature and matric water potential in controlled laboratory experiments.
The present study represents a significant step forward in understanding the effects of warming in Arctic ecosystems on soil fungi. Efforts will be made to answer the following research questions after the completion of the study: what effect does warming have on soil fungal community structure? Are the growth rates of soil fungi affected by warming? Are the enzyme activities of fungi present in Arctic soil affected by warming?

Compositional shifts in arctic ectomycorrhizal fungal community in response to long-term increased snow depth in Northern Alaska

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Recently, various climate-induced changes have been observed in arctic tundra ecosystems, e.g., shrub expansion, resulting in reduction in albedo and greater carbon (C) fixation in aboveground vegetation, as well as, increased rates of soil C mobilization by microbes. Importantly, the net effects of these shifts are unknown, in part because our understanding of belowground processes is limited. Here, we focus on the effects of increased snow depth, and as a consequence, increased winter soil temperature on ectomycorrhizal (ECM) fungal communities in dry and moist tundra. We analyzed deep DNA data generated from soil samples taken at long-term (18-year) snow fence experiments at Toolik Lake, Alaska. In the dry tundra, both community richness and composition were significantly altered. There was a decrease in richness in *Tomentella*, *Inocybe* and taxa with contact, short- and medium-distance smooth hyphal exploration types. On the other hand, richness of *Cortinarius*, including species with abilities to scavenge for recalcitrant forms of nitrogen, did not change. In the moist tundra, only community composition changed, richness did not, and there were strong OTU-specific responses to the altered conditions. Our findings indicate that ECM fungal community responses to long-term increased snow depth are dependent on taxa and on tundra types.

Soil warming changes litter chemistry and fungal community composition but not decomposition rate

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Plant carbon (C) is stabilized in soils as microbial biomass, with leaf litter decomposition representing an important source of energy and nutrients for soil microbes in temperate forests. Global change stressors such as increased temperature may change the rate at which litter is decomposed or may change litter quality in terms of the types of compounds that are preferentially consumed. Changes in decomposition rate or litter quality will affect long-term C stabilization in soils to the extent that these changes are reflected in microbial biomass; through either the amounts or kinds of C compounds produced by soil microbes as they consume litter resources. We used a long-term soil warming experiment, established in 2003 at the Harvard Forest LTER, Massachusetts, USA, to examine the effects of increased temperature on litter decomposition and the litter fungal communities, with the hypothesis that warming would increase decomposition rates and consumption of labile C resources, and increase relative abundance of active saprotrophs. Mixed tree species litter bags were installed at the experiment in autumn 2010, and allowed to decompose for one or two years. After harvest we measured mass loss, extracellular enzyme activities (EEA), 'total' and 'active' fungal community structure by sequencing ITS2 from rDNA and rRNA, and litter chemistry by pyrolysis-gas chromatography/mass spectrometry. Soil warming had no effect on litter mass loss after one or two years. After two years warming caused shifts in EEA characterized by decreased cellulolytic EEA and increased ligninolytic EEA, and notably, a reduction in the ratio of beta-glucosidase to polyphenol oxidase activity (BG:PPO). Warming altered fungal community composition in both years. Specifically, after two years of decomposition warming favored ectomycorrhizal fungi (ECMF) and disfavored some clades of litter saprotrophs. The relative abundance of active ECMF explained 75% of variance in BG:PPO. Decreased BG:PPO suggests that though warming did not increase litter decomposition rate as hypothesized, it did alter litter quality by changing the types of compounds that were consumed by microbes. Expected increases in decomposition rate with warming may be mitigated by increased abundance of ECMF, which compete with saprotrophs for nitrogen. Altered EEAs were concomitant with a shift in fungal community composition, which may result in a shift in the types of C compounds that are stabilized in warmed soils.

Catch me if you can – The impact of mycelia-based dispersal on predator-prey interactions and biodegradation of soil contaminants

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Knowledge to predict the soil's ability to degrade a chemical and to cope with an ever increasing variety of contaminants is still highly insufficient. At biogeochemical interfaces (BGI) biodegradation is aggravated by the concurrence of restricted bacterial mobility and retarded transfer of organic contaminants. In order to deal with heterogeneous soil environments mycelial fungi have developed a unique network-based growth form. Unlike bacteria, hyphae spread efficiently in soil, penetrate air-water interfaces and cross over air-filled pores between the bacteria and contaminants in the vadose. Mycelia thereby act as effective dispersal networks for both undirected and targeted mobilization of contaminant degrading bacteria ('fungal highways') and may positively influence degradation of contaminants. Nothing however is known about the role of mycelia-based dispersal on predators-prey interactions and concomitant effects on the biodegradation of soil contaminants. Here, we investigated the impact of dispersal networks on the dispersal of the predator *Bdellovibrio bacteriovorus* 109J and its effect on the biomass of the phenanthrene (PHE) degrading *Pseudomonas fluorescens* LP6a (as prey) and assessed its impact on PHE degradation. We thereby challenged the hypothesis that the presence of dispersal networks leads to: (i) improved bacterial dispersal of both prey and predator bacteria, (ii) scouted travel by predator and a reduction of the biofilm of the prey bacteria due to predation, and (iii) an increased contact ratio between prey and predator with a positive effect on the biodegradation in the system. We designed a laboratory microcosm mimicking a PHE release from a PHE source to a model BGI (agar surface) in presence of glass fibres (as a laboratory mimic for mycelia) as facilitators of the dispersal of the unless poorly motile predator and prey bacteria, respectively. The presence of the glass fibres resulted in an (i) increased spatio-temporal spreading of both the predator and the prey bacteria, (ii) an increased dispersal of the predator in presence of the prey and a significantly reduction of the prey biomass, and (iii) PHE degradation increase releative to the amount of predators in the system. Our data suggest that networks may promote the formation of an adapted microbial population that will degrade contaminant molecules but at the same time increase the possibility of predation and due to this fact also affects the efficiency of biodegradation.

16S rRNA gene family based microbial typing of rhizospheric communities of a native legume *Alysicarpus vaginalis* (L.), fam. Fabaceae.

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A study was undertaken to understand microbial diversity associated with rhizospheric samples of a native legume *Alysicarpus vaginalis* (L.), found at an exhausted Limestone-dolomite mined out area at Purnapani (N 22º24'47.28'' E 84º52'11.4''), Sundargarh district, Odisha, India. *Alysicarpus* sp., is one of the early colonizers of degraded soil systems and is characterized by its annual, seed propagated, creeping habit and shade intolerant nature. For typing of the rhizospheric microbial community at spatial and temporal scales, PCR amplifications, clonal library preparations, restriction digestion based binning by ARDRA and Sanger sequencing based characterization of microbial 16S rRNA gene family was done. Clonal library based Sanger sequencing results were validated by next generation sequencing based metagenomic survey of soil DNA using V3 and V6 regions. Highly diverse communities of bacteria with members belonging to 24 bacterial classes were detected in varying abundances. More than 1500 bacterial OTUs were observed which included members of Chloroflexi, Chlorobi, Tenericutes, Deinococcus-Thermus, Elusimicrobia, Spirochaetes, and Nitrospirae bacterial classes. A bootstrapped RAxML III tree generated using unique 16S rRNA gene sequences with reference sequences at >97% sequence similarity indicated that bacterial community structures of *A. vaginalis* rhizospheres shifted towards those observed in forest soil type. Results from quantitative PCRs suggested that the primers were specific, but not sensitive enough to detect variability in amounts of bacterial DNA targets from samples originating in the study area.

Looking for the core microbiome of the main types of soils in Russia

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Widespread adoption of high throughput sequencing unlocks unique possibilities to assess general mechanisms behind the development of soil microbiomes on a global scale as well as to model their temporal variation. The attention to the driving-factors of soil microbial diversity keeps growing, yet most publications describe soils of Northern and Southern America and Western Europe, while Russian soil has remained drastically understudied so far, though it exhibits most known soil types, including the most fertile ones – the chernozems. This is the first work to describe microbial diversity of six major soil types (based on 4 samples per type): Albeluvisols Umbric, Chrenozems Chernic, Phaeozems Albic, Kashtanozems Haplic, Solonetz Humi?, Cambisols Chromic (WRB classification, 1998). We used multiple matrix regression based on Mantel permutations to reveal the relationships between community composition and different agrochemical properties of soils. The analysis showed that despite the immense diversity exhibited by the microbiomes we could still model their pair-wise differences fairly well given agrochemical data such as pH and K (model R squared ~ 0.61, p-level < 0.0005). Along with that we incorporated a classical system of quantitative soil type classification – commonly used in Russian soil science – that allowed us to reveal a trend between soil fertility and its quantitative properties. We observed several bacterial groups in the 16S libraries that were present in all soil types, though we found no single completely ubiquitous species. Bradyrhizobiaceae, being present in 95% of studied samples, were the most ubiquitous bacteria of all. Not only this work provides a new perspective of Russian soil microbiome as of a significantly understudied object, but it also provides a sneak peek of the ways to use soil microbiome structure to infer soil quality.

The influence of salinity on saprotrophic fungi and bacteria in soil

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Saline soils are widespread and salinization is increasing. Microbial activity is lower in saline soils than in non-saline soils. However, beyond this crude insight little is known on salinity's influence on the ecology of bacteria and saprotrophic fungi. Changes in the relative importance of fungi and bacteria are thought to have implications for soil C and nutrient cycling. Pure culture studies suggest that fungi are more resistant to low water potentials and should cope better than bacteria with high salinity. However, results on bacterial and fungal responses to salinization are hitherto ambiguous, including reports of both lowered and higher relative abundance of fungi.
We investigated (i) if fungi or bacteria are more resistant to acute salt exposure, (ii) the recovery of bacterial and fungal growth rates after exposure to salt and (iii) the relative abundance of fungi and bacteria along natural salinity gradients. Firstly, we exposed a non-saline soil to salts associated with soil salinization and compared the degree to which fungal and bacterial growth rates were inhibited by acute salt exposure. Secondly, soil microcosms with plant litter and different concentrations of NaCl were monitored for changes in microbial growth rates over time. Thirdly, two natural salinity gradients were assessed encompassing a wide range of electrical conductivities from 0.1 dS m^{-1} to >10 dS m^{-1}.
Bacterial growth was more sensitive than fungal growth to salt exposure. Salt exposure also initially reduced growth rates over time; however, at high salt application rates, fungal growth rates recovered faster than bacterial, suggesting a better ability of fungi to function at high salinity. A link between reduced bacterial growth and stimulated fungal growth was observed during the succession on plant material additions. As such, changed bacterial and fungal balance could be partly due to competitive interactions. Growth assessments indicated that both groups decreased similarly toward higher salinities along the natural gradients. However, microbial PLFA and ergosterol concentrations both suggested higher relative abundance of fungi towards higher salinity and the PLFA composition was linked to salinity. Our results show that fungi can be more resistant to increased soil salinity than bacteria. This asymmetrical impact by salt on the microbial community could lead to a shift in the microbial structure and soil functioning in response to rising salt concentrations.

Potential activity of microbial community in the Biological Soil Crusts

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Biological soil crusts (BSC), which consist of phototrophic and heterotrophic microorganisms (cyanobacteria, green algae, bacteria, microfungi), are an important component of the land surface of the arid and temperate regions around the world (West 1990). Through their ecosystem functions, these microbial communities affect multiple physico-chemical properties of the soil on which they occur. As such they generally prevent soil erosion, keep soil water and increase fertility (Belnap and Lange 2001). In addition to these functions, cyanobacterial and microalgal communities (as first colonizers of high-mountain soils) affect nutrient availability for vascular plants, and water and nutrient status of plants (Řeháková et al. 2011). The presence of microbial crusts can strongly promote the occurrence and development of the vegetation (Schmidt et al. 2008). However, microbial communities in BSC can only supply the environment with essential nutrients when they are in an active state. The aim of this study is to quantify photosynthetic and heterotrophic activity of BSC in high mountain cold desert of Tibetan Plateau (W Himalaya, Ladakh) and in High Arctic (S Svalbard). We hypothesize that the most important factors which influenced the metabolic activity of BSCs of microbial communities of BSCs are the effect of temperature and moisture. We were measured BSC production and consumption rates of CO2 and O2 in situ and directly in the field under different temperatures and moisture levels and at light and dark conditions to separate metabolic activity of heterotrophs and autotrophs from each other. We also measured other significant environmental characteristics such as soil temperature, soil moisture, amount of photosynthetic active radiation, and amount and stoichiometry of soil nutrients, to propose a numerical model that may be used to estimate the overall metabolic activity of BSCs.

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How do soil microbial communities react on droughts and heavy rainfall events?

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The predicted increase in incidence and duration of weather extreme conditions like drought and rainfall events as caused by climate change may directly affect soil microbial community structures and their physiological capacities. This might feedback ecosystem processes like biogeochemical cycles, nutrient cycling and decomposition processes in soils.
In a four year-long field experiment, we aim to investigate the response of microbial communities on drying and rewetting cycles. A field experiment has been set up in a temperate beech forest in the Rosalien Mountains in south-east Austria at about 640 m above sea level with a mean annual temperature of 6.5°C and an annual precipitation of 800 mm. Extreme weather events are simulated with roof installations and an irrigation system from May to October. Soil samples are taken monthly before and after irrigation events that follow after drought periods of four and eight weeks, respectively. Total viable microbial biomass, microbial community structures, and possible changes therein are first analyzed with phospholipid fatty acid analysis (PLFA). Based on these signature molecules the isolated microorganisms are classified into major microbial groups (Fungi and Bacteria) and sub-groups (like Gram+, Gram- Bacteria, and Actinobacteria). Furthermore, abundances and activities of soil microorganisms are assessed by DNA- and RNA-based analysis. Enzymes responsible for the present microbial processes in the soil are also quantified. We will subject the samples reflecting the most pronounced changes to metaproteomics (analysis of the expressed proteins). The application of metaproteomics and enzyme activities will enable profound community structure and function profiling.
Here we present the response of the microbial communities to the simulated and natural weather conditions estimated by PLFA analysis and nucleic acid based analyses.

Response of Soil Fungal Communities to Extended Drought

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Soil fungal communities affect nutrient availability, plant growth, and the carbon budget of terrestrial ecosystems. However, how such communities react to changing environments is not well understood. In this project we examine the impacts of drought on fungal communities in European temperate grassland soils. Our experimental systems were Terrestrial Model Ecosystems (30cm x 40cm soil cores) which were kept under controlled conditions, and exposed to different watering treatments over the course of 3.5 months. Changes in fungal diversity and community composition were analyzed and correlated among treatments (30%, 50% and 70% of the maximum water holding capacity of the cores). Illumina high throughput amplicon sequencing of the ITS rDNA fungal barcode marker yielded >3.000 fungal operational taxonomic units in a total of 96 soil cores. The Shannon diversity of soil fungi was not significantly influenced by different watering treatments, although we could see an effect of watering in interaction with above ground plant mass on the soil cores. In dry conditions fungal diversity was higher in soil cores with more above ground plant mass. We also report changes in taxonomic composition of the fungal communities under different water regimes, and identify operational taxonomic units that are most influenced by drought conditions.

Preliminary data of soil nematode communities along a rainfall gradient in Costa Rica.

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We characterized soil nematode communities at four locations in grass-dominated patches along a rainfall gradient from Bagaces, Guanacaste (<100 mm) to Golfito, Puntarenas (6000 mm) and compared soil properties (e.g. water content, organic C, total N) and nematode community composition and diversity. Soil samples were collected from each site from a depth of 0-10 cm. A 100 g soil sample of each replicate was used for nematode extraction, using the Baermann funnel method. The nematodes from each sample were collected and identified according to order and family, using a compound microscope. Nematodes were classified based on morphology in the following trophic groups: (1) bacterivores; (2) fungivores; (3) plant parasites; and (4) omnivores/predators. The results of this study will provide insight into the potential impact of climatic variability and changes in the rainfall regime on soil nematode community size and structure in these ecosystems.

Active microbial community resilience in disturbed soil with nitrogen source enrichment and nitrification inhibitor

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Ecosystem stability following disturbance comprises both resistance (the ability of the system to stay unchanged after disturbance) and resilience (the ability of the system to recover after disturbance). A source of disturbance for soil habitats is nutrient enrichment through anthropogenic activities. Although is known that fertilizers modify soil properties, changes in structure and resistance and/or resilience of microbial communities across enriched habitats remain unclear. Therefore, to have insights on microbial resilience and resistance, we evaluated the soil active microbial community after the disturbance with dicyandiamide (DCD) and different nitrogen sources in short-term experiment in no-till corn with the following treatments: control; swine slurry (SS) at 49 m? ha^{-1}; swine slurry amended DCD (SS+DCD) in a rate of 10 kg ha^{-1} and mineral fertilizer (NPK). Soil samples were evaluated before and after 3, 6, 12, 25 and 50 days to experiment installation. The 16S rRNA transcripts was amplified and sequenced by PGM ION TORRENT platform and beta and alpha diversity of the samples were analyzed using scripts in QIIME. The Principal Coordinates Analysis (PCoA) showed that all microbial communities pre-experiment were similar but after 3 days the microbial communities from soils that received addition of SS with or without DCD differed from those that received NPK or was unfertilized. After 6 days, the dissimilarity between microbial communities from soils with addition of SS and SS+DCD decreased. This dissimilarity remained decreasing at each time of sampling and finally the microbial community returned to original status at day 50 showing a resilient effect in a short-term experiment. The use of DCD did not affect the soil microbial communities. In addition, no differences between microbial communities from the control and NPK were detected within either time of sampling. The effect of SS on alpha diversity was in agreement with beta diversity analysis. Calculations Chao, Faith's phylogenetic diversity PD and Shannon diversity index were initially similar before treatments but diverged after treatments. All indices showed low diversity in those soils with addition of SS (with or without DCD) at 3 days of experiment. After the initial days with organic amendment, the diversity returned to the original status. Altogether the results indicated that soil microbial communities were resistant to NPK addition but resilient to addition of slurry swine.

Genetic diversity and catabolic activity profiles of rhizosphere bacterial communities during dry and wet seasons in a Solonchak grassland, Hungary

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Genetic diversity and community level physiological profile (CLPP) of rhizosphere samples originated from four different plant communities in Solonchak grassland located in Kiskunság National Park were investigated following a sampling in June and September 2014. This saline sodic soil is typically dry during summer and wet during winter, however several dry/wet events may occur during the vegetation season. The soil was very dry in June while it was wet or even waterlogged in September. The soil physical and chemical properties were quite different at the four sites: the *Lepidio crassifolii-Camphorosmetum annuae* site had the highest salt content and the highest pH and the lowest humus content followed by *Lepidio crassifolii-Puccinellietum limosae* then the *Artemisio santonici-Festucetum pseudovinae* and the highly diverse *Achilleo-Festucetum pseudovinae* shortgrass pasture. CLPP of bacterial communities was assessed by MicroRespTM method using 15 different substrates, including monosaccharides, amino-acids, carboxylic acids and an aromatic acid, while Denaturing Gradient Gel Electrophoresis (DGGE) was used to compare the genetic diversity. Multivariate statistics revealed that samples according to sites were well separated from each other both in June and in September by CLPP and DGGE and within each site the samples were strongly differed according to the sampling date by DGGE. The observed differences in DGGE profiles was far greater according to the sampling dates then the spatial differences within each site suggesting a significant shift in bacterial communities during the dry and wet seasons. The genetic profile as well as the catabolic activity profile of the samples was influenced by soil properties, mainly the salt content, alkalinity and the humus content and the moisture conditions. Members of the phyla Acidobacteria, Bacteroidetes, Proteobacteria and Actinobacteria were identified according to the DGGE.
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Spatial distribution of microscopic fungi under old environmental burdens

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Soil microscopic fungi are can occur under wide range of soil pH. This ecological factor limits their existence in the environment as well as such microorganisms affect pH values through their own metabolic activities.
The spatial distribution of soil microscopic fungi at three different contaminated sites originated by mining activities was observed. All sampling points were localized by GPS. The spatial distribution of microscopic fungi in vertical and horizontal direction was observed.
Substrate samples from old environmental burdens were enriched by various heavy metals and potentially toxic elements such as As, Cd, Cu, Zn, Sb highly exceeding their limits. The pH values of substrate samples varied from ultra acidic (pH 3.12, Banska Stiavnica-Sobov) to neutral (pH 7.23, Zemianske Kostolany) and very strongly alkaline (pH 9.4, Slovinky). The percentage organic content was very low in all substrates varying from 0.3 (Slovinky) to 3.54 (Zemianske Kostolany). The mycocoenosis of substrates was consisted mainly from species from genera *Penicillium* representing 25 species with most frequently occurred *P. chrysogenum* var. *chrysogenum* and *Aspergillus* representing 14 species; *A. niger* was isolated from all samples. *Eupenicillium* (7 species), *Trichoderma* (6 species), *Neosartorya*, *Paecilomyces*, *Talaromyces* (each genera for 5 species) a *Cladosporium* (4 species) were frequently represented. The group Zygomycota/Mucoromycotina is marginally represented (8 genera, 17 species).
Substrates with long-lasting contamination represent sources of new species of soil microscopic fungi adapted to specific ecological conditions even on their genetic level. From all 109 genera and 192 species of identified soil microscopic fungi isolated from contaminated substrate samples, 14 new species (*Alternaria triticina*, *Bionectria ochroleuca*, *Bjerkandera adusta*, *Chrysosporium queenslandicum*, *Clonostachys pseudochroleuca*, *Exophiala psychrophila*, *Lewia infectoria*, *Metarhizium robertsii*, *Neosartorya laciniosa*, *N. tsunodae*, *Phoma macrostoma*, *Purpureocillium lilacinum*, *Trichoderma rossicum* and *Phlebia acerina*) were recorded.
Significant representation of phytopathogenic species is the response of plant community weakened by heavy metals and toxic elements presented in the substrates.
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Bacterial response to rainfall and draught cycles in desert soil

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Vast regions of the Earth's surface are arid, characterised by sparse vegetation interspaced by dry and barren soil surfaces. The effect of hydration on soil microbial populations is being debated for many years and different scenarios were suggested. Yet, in-depth analysis of the changes in desert soil bacterial communities starting from the rain pulse through the desiccation of moisture from the soil, were never reported, bared a few scattered snapshots of soil microbial profiles during hydration or desiccation events.
We predicted that like macro-organisms hydration would supply microorganisms with much-needed water and increase their diversity and abundance. Whilst, desiccation would eliminate those who cannot survive water shortage and reduce both diversity and abundance of the bacterial community. To test our predictions we closely followed major rain events and the subsequent soil desiccation in the field and in simulated hydration of arid soil columns. We thus aimed to link bacterial diversity and interactions with soil water content.
Detailed monitoring of a natural microbial community in desert soil following rainfall revealed a remarkable decrease in species richness and diversity that were gradually restored during soil desiccation. Our controlled experiments showed that the changes in abundance and diversity wane when desiccation was rapid, suggesting that not only the amount of rain but also the air temperatures would impact the soil microbial community
We suggest that after rainfall, bacteria are involved in complex community dynamics; as the moisture in the soil increase gaps between soil particles are bridged by water, enabling interactions and competition between formally separated communities leading to antagonistm and a decrease in the overall bacterial diversity. However, when the soil desiccates the pores are again segregated and unique communities are established in each microhabitat thus stimulating bacterial dispersal.

Investigating the ecophysiology of the ubiquitous Acidobacteria in the dynamic soil environment

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Acidobacteria represent one of the most abundant and ubiquitous bacterial phyla across terrestrial environments. The phylum harbors extensive phylogenetic diversity spanning 26 subdivisions, yet only few species have been cultivated and little is known about their ecological function. We sought to better elucidate the ecophysiology and therefore the success and prevalence of Acidobacteria in terrestrial ecosystems by combining genome and growth-based analyses. We are analyzing 23 acidobacterial genomes that span subdivisions 1, 3, 4, 8 and 23. The genomes are circular chromosomes with size ranging from 2.7 to 9.9 Mb; although many are considered draft genomes, they are estimated to be at least 95% complete. Phylogenetic analysis of the 16S rRNA gene and concatenated protein tree from our genome data confirms the monophyletic nature of the phylum. Efforts are underway to better understand the genomic features of acidobacteria as well as define the acidobacterial pan genome. Our initial genome analyses reveal traits that provide physiological and metabolic versatility, making them well adapted to the fluctuating soil environment. For example, select strains in subdivisions 1 and 3 appear to be capable of using various carbon sources, from monomers to polysaccharides, and harbor an abundance of conserved gene clusters in the carbohydrate-active enzyme family. Strains contain either 1 to 2 copies of the 16S rRNA encoding gene, which is suggestive of an oligotrophic lifestyle. Our genomic analyses identified genes in several strains that encode for low as well as high-affinity cytochrome oxidases, suggesting the potential for adaptations to changing O_2 concentrations. We have uncovered a series of genomic features which we hypothesize allows them to adapt to the ever changing soil environment.

Effect of Cosmetic Based Nanowaste on Sludge and Soil Microflora

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The release of nanowastes from nanomaterial based commodities is not a hypothetical situation but is already occurring. But, the estimation and the prediction of nanoparticle quantities are the major issues nowadays. Since, they have been found to disturb the ecosystems by causing harm to microbial population, plants and animals. Several cosmetic companies use nanoparticles in their formulation. However, the number of cosmetics involving nanoparticles is not clearly known therefore it is an utmost importance to focus the study towards nanowastes and its impact on environment. The toxicity of TiO_2 nanoparticles released from a cosmetic formulation was studied in this work. Dynamic light scattering (DLS), inductive couple plasma atomic emission spectroscopy (ICP-AES) and scanning electron microscope energy dispersive spectroscopy (SEM-EDS) was used to analyse the TiO_2 nanoparticles in sludge, and the soil amended with TiO_2 containing sludge. FTIR spectra determined the biochemical alterations produced in sludge and soil due to the presence of TiO_2 nanoparticles. For testing nanotoxicity in sludge and soil microflora, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and fluorescent based live-dead assay was performed. The results proved the detrimental effect of TiO_2 nanoparticles on microflora. Our study concludes that there is an immediate attention required to prepare guidelines for the use of such commercialized nanoparticle-based products.

Response characteristics of soil microorganism to permafrost degradation in the upstream regions of the Shule river basin, Qinghai-Tibetan Plateau

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Permafrost degradation significantly influences the alpine ecosystem of the Qinghai-Tibetan Plateau (QTP), including both vegetation characteristics and soil properties. However, there is a paucity of knowledge on the soil microorganisms. In this study, the Illumina MiSeq and Biolog Eco-plates (4ºC and 25ºC) were utilized to investigate soil bacterial community structure and function from four principal permafrost types: sub-stable permafrost (SSP), transition permafrost (TP), unstable permafrost (UP) and extremely unstable permafrost (EUP) in the upstream of the Shule River basin. The results showed that Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes were the predominant phyla in all four permafrost types soil. However, the relative abundance showed different patterns : the relative abundance of Proteobacteria decreased in the order: SSP>TP>UP>EUP, whereas the Actinobacteria abundance increased. Particularly, the ratios of Actinobacteria/Proteobacteria increased significantly along with permafrost degradation, which may could use as a sign of permafrost degradation. Partial RDA analysis showed that the soil properties and vegetation characteristics together determine the bacterial community structure in this area. Non-metric multidimensional scaling (NMDS) analysis revealed differences in soil bacterial functional diversity as a function of temperature between the four permafrost environments. At 25ºC, soil total nitrogen, vegetation diversity index (H') and species richness index (S) were positively correlated with bacterial utilization of 31 different carbon sources. In contrast, grain size distribution determined by the proportion of coarse sand (PCS) was negatively correlated with microbial activity at 4ºC. The results of this study indicate that patterns in soil bacterial community structure and function can be clearly discerned across permafrost types. likely due to the difference in soil properties and vegetation characteristics.

Unexplored Biodiversity and Function of arbscular mycorrhizal fungi on the Tibetan Plateau

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The diversity of arbuscular mycorrhizal fungi (AMF) on the Tibetan Plateau remains largely unexplored, and their contribution to soil aggregation can be important in understanding the ecological function of AMF in alpine ecosystems. Two experiments were conducted in the present study. In the first experiment, we took sample along an elevational transect (4,149 to 5,033 m a.s.l.) from the west slope to the east slope of Mount Mila. Roots of *Kobresia pygmaea* C.B. Clarke (dominant sedge species) and *Carex pseudofoetida* Kük. (sub-ordinate co-occurring plant species) were analyzed for AMF diversity. The second experiment was conduced in July 2012. We selected forest, grasslands and arable fields from three sampling sites with a distance of at least 20 km apart on the Southeast Tibet to explore the influence of land use change on AMF community and soil C sequestration. A structural equation model was built to explore the contribution of AMF and other biotic factors to soil aggregation. Sedges harboured abundant AMF communities covering seven families and some operational taxonomic units are habitat specific. The relative abundance of the two sedges contributed largely to soil macroaggregates, followed by extraradical mycorrhizal hyphae (EMH) and total glomalin-related soil protein (T-GRSP). The influence of plant richness was mainly due to its indirect influence on T-GRSP and EMH. There was a strong positive correlation between GRSP and soil total carbon and nitrogen. AMF diversity, HLD, T-GRSP and total C in arable lands were significantly lower than those in forest and grasslands. The AMF community composition differed significantly among land use types. Land use change affected AMF community and HLD through indirect path mediated by soil pH. Land use mainly influenced macroaggreates and total C through indirect path mediated by affecting HLD and EE-GRSP. Our results demonstrate that AMF contribute to soil aggregation and thus may have the potential to greatly influence C and N cycling in alpine grasslands. Proper management is crucial for the sustainability of the alpine ecosystems.

Microbiological indicators to evaluate soil quality of degraded areas in Southern Italy after compost addition

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Microbial communities provide several regulation ecosystem services, which ensure soil quality and fertility. In fact, they adapt promptly to environmental changes by varying their activity and by increasing the growth of populations that can adapt to them. The structure (e.g. cell abundance and diversity) and functioning (e.g. viability and activity) of natural microbial communities under different stress conditions can be used ad useful indicators of soil quality state. Soil quality assessment is a crucial step in characterization of areas affected by land degradation processes and in recovery strategies. In this work we applied microbiological measurements to assess soil quality of three different sites, located in Taranto Province (Southern Italy), affected by land degradation processes, such as organic matter decline and contamination (PCB and heavy metals). Compost, produced from selected municipal organic waste was added to the soils in order to improve the their quality state.
Soil samples were collected before and after compost addition and both microbial and chemical analyses were carried out in order to evaluate the soil quality state at each site at different times. For this purpose, the microbial indicators evaluated were bacterial abundance (DAPI counts), cell viability (Live/Dead method), dehydrogenase activity (DHA) and soil respiration. At the same time, the main physico-chemical soil characteristics (organic carbon, available phosphorous, total nitrogen, carbonate and water content, texture and pH) were also measured. Moreover, in the contaminated soil samples, PCB and heavy metals (e.g. Pb, Se, Sn, Zn) were analysed by GC-MS and ICP-MS, respectively.
The overall results showed that the bacterial structure and functioning were affected in different ways by the organic carbon addition with compost and contaminant occurrence (organic or inorganic compounds). The compost treatment improved soil quality in terms of an increase in organic carbon content 7 months after its addition in all the three survey sites. However, in the contaminated site the increase of bacterial activity, observed 4 months after the compost treatment was not related to the organic carbon content, but it was presumably due to the general decrease in inorganic and organic contamination levels.

Spatial metrics indicate bacterial degradation benefits from mycelial networks

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The turnover of natural or anthropogenic compounds by microbial activity has a strong impact on soil ecosystems, e.g. affecting soil health, trace gas production, carbon storage, or nutrient provision. Concerning organic contaminants, bacterial degradation is an important ecosystem service. In water-unsaturated soils, however, this service is often impeded by a low bioavailability of contaminants to degrading bacteria. Experimental and modelling studies have shown that mycelial networks may considerably improve this bioavailability if they provide continuous liquid films that facilitate bacterial dispersal. Since many mycelial fungi are well-adapted to typical soil heterogeneities, fungal networks as bacterial dispersal infrastructure are promising for the development of novel resource-efficient bioremediation methods.
In particular, simulation studies highlighted that the spatial configuration of such dispersal networks is a key factor for improving bacterial degradation. As these spatial configurations are typically complex and not precisely known, we searched for characteristics that indicate the networks' impact on biodegradation performance. To this end, we used a spatially explicit microbial model to randomly create manifold dispersal network configurations and simulate the associated bacterial degradation of organic compounds. We investigated various aggregated spatial metrics for characterizing the network configurations, and identified appropriate indicators of biodegradation performance. Our results show that several single metrics are suitable for rough assessments of biodegradation performance, and that well-chosen combinations of two metrics can serve as very good indicators. Specifically, we found that a high coverage, i.e. dense and widespread networks, and a direct accessibility to degrading bacteria will substantially benefit contaminant degradation. Therefore, when making use of fungal-bacterial consortia in contaminated soil management, strategies to measure, stimulate or sustain these characteristics need to be developed. Beyond the relevance for bioremediation, these network characteristics are likely to determine the impact of fungal networks on compound turnover in many soil ecosystems, especially when abiotic conditions restrict bacterial motility.

Use of molecular techniques to characterize the microbial communities for soil ecology assessment in degraded sites

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Traditionally, the identification and characterization of microbial communities in contaminated soil and water were limited to those microorganisms that are cultivable. The application of molecular techniques to study microbial populations at contaminated sites, without culturing, allowed to the discovery of previously unrecognized microorganisms as well as to the knowedge of the microbial diversity, which can play a key role in bioremediation strategies of contaminated sites. Polymerase chain reaction (PCR) analyses, among of several bacterial profiling techniques, are extremely useful in characterizing microbial community structure in contaminated soils.
In this work we used PCR to identify microbial communities in soils affected by pollution or organic matter depletion located in degraded sites in Southern Italy. Moreover, PCR analyses and complementary bacterial investigations were carried out on these soils after the application of different soil recovery treatments (compost addition/ plant assisted bioremediation) in order to evaluate modifications in microbial communities and consequently assess soil quality restoration.

Soil dehydrogenase activity under the presence of some exobiotics. A toxicity index is proposed.

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Soil dwelling organisms are responsible of many chemical transformations which are linked directly to quality and fertility of soil. The human activity makes that more and more strange substances (xenobiotics) reach the superficial layers of soil and concerns about the safety of these products have been raised. Risks assessment has become a common practice, especially in the framework of agricultural procedures where pesticides are willfully delivered to kill organisms. The assessment of the toxicity that pesticides may have on soil environment is crucial since the sustainability of the whole system could be endangered. In this work, the toxicity of some xenobiotics (Metam Na, Oxyfluorfen, Glyphosate, Metribuzin and Diuron) has been evaluated through the determination of the hydrogenase activity (ADH). A toxicity index is proposed to compare the different degrees of toxicity among the substances tested. For this reason a toxicity threshold has been created with the slopes of the fitted lines of the data assuming that the maximum toxicity (10) is for the slope of the biocide $HgCl_2$ and de minimum (1) is given by the ADH obtained with the soil without pesticides. The results show that high concentrations of these pesticides have an inhibitory effect in the dehydrogenase activity, affecting negatively the soil microbial activity but within a wide range of toxicity.

A pyrosequencing-based metagenomic study of microbial communities during co-composting of creosote-impregnated wood and green wastes

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Co-composting of polluted matrices with green wastes has shown its bioremediation potential and it is compliant with the EU Directive 1999/31/EC, which promote the reduction of biodegradable organic matter being sent to landfills. In our pilot-scale test, creosote-impregnated wood (overall PAH content of 26498 mg kg^{-1}) was the target material while grass cuttings (GC) and pre-treated broiler litter (BL) were used as bulking agents. Besides assessing the effectiveness of the two composting processes in term of contaminant and toxicity reduction, the work was aimed to study the shifts occurring in microbial community structure through phosholipid fatty acid analysis (PLFA) and 454-pyrosequencing of 16S rRNA and ITS gene sequences. At the end of 240 days of incubation contaminant depletion was 97 and 81% of their original concentration in GC and BL compost, respectively. The time course of PAH biotransformation was accompanied by a significant drop in toxicity towards the luminescent bacterium *Vibrio fischeri* and phytotoxicity (barley - *Hordeum vulgare* L. - seeds germinability assay). The value of the fungal/bacterial PLFA ratio remained rather low in both treatments, with the only exception of BL compost in the maturation phase. Total microbial biomass production as inferred by the sum of PLFA markers paralleled the time course of contaminant removal, peaking and declining earlier in GC than in BL compost. Interestingly, distinct and rapid changes in microbial community structure were observed in both composting processes, as a consequence of temperature profile, nutrient and contaminant depletion. An extensive proliferation of Gram-positive bacteria, mainly Firmicutes (Bacilli) and Actinobacteria, coincided with the highest extent of PAH degradation in GC compost, whereas Gram-negative (Proteobacteria) dominated in BL co-substrate throughout the whole incubation. As for the fungal community, members of division Ascomycota (Saccaromycetes and Pezizomycetes) were the most abundant in the crucial phases of PAH degradation.

Monitoring the impact of bioaugmentation with a PAH-degrading strain on different soil microbiomes using pyrosequencing

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Bioremediation is an environmental friendly approach for the cleanup of contaminated soils with complex mixtures of organic pollutants as polycyclic aromatic hydrocarbons (PAH). In aged contaminated soils, the perturbation caused by the contamination on native microorganism could limit the biodegradation of recalcitrant compounds, there bioaugmentation would be a treatment option.The present work studied the effects of inoculation with the strain *Sphingobium* sp. (AM) on a soil chronically contaminated with a petrochemical sludge (IPK+AM), containing 10 priority PAH and aliphatic hydrocarbons in high concentration, 576 mg/kg and 207 mg/kg respectively. Inoculant behavior was compared on pristine soil artificially contaminated with 2000 mg of phenanthrene/kg soil (Phe+AM) and pristine soil (PS+AM). AM was isolated from a chronically contaminated soil, is capable of degrading phenanthrene, anthracene, fluorene and pyrene; the partial sequencing of its genome showed PAH and aliphatic degradation complete pathways.
Microcosms were inoculated with $1.4x10^8$ cfu/g dry soil and incubated at 20±2°C and 21% humidity for 63 days. Non-inoculated microcosms of each soil were used as control. During incubation the PAH concentration (GC-FID), the number of PAH-degrading bacteria, dehydrogenase activity, and the genetic diversity (454-Pyrosequencing) of bacterial soil community were analyzed.
Inoculation increased PAH degrading bacteria counts and phenanthrene degradation in Phe+AM, although in IPK+AM no effect on the degradation of any PAH was observed. In Phe+AM the dehydrogenase activity increased after 7 days of incubation, while IPK+AM and PS+AM didn't show this stimulatory effect. The pyrosequencing data analysis, revealed that after 14 days of incubation, populations belonging to the genus *Sphingobium* became dominant in Phe+AM and IPK+AM (relative abundance 27.1% and 11.5% respectively). Hill numbers (D0, D1 and D2) indicated that inoculant produced an initial reduction on microbial diversity only in contaminated microcosms (Phe+AM and IPK-AM). However, at the end of the incubation, Phe+AM and IPK-AM achieve greater richness and diversity than their respective controls. The highly specialized strain *Sphingobium* sp. AM, used as inoculant in soil with complex mixtures of PAH, could establish and produce changes in the diversity of the bacterial communities, but these changes didn't cause any improvement in global degradation efficiency on chronically contaminated soil.

A novel bioaugmentation approach for PAH-degrading bacteria in soil: Adaptability as assessed by molecular biology techniques

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Polycyclic aromatic hydrocarbons (PAHs) belong among organic contaminants that accumulate in the environment mainly as a result of human activities. Due to their well-known negative effects on human health, methods aiming at their removal from the environment are intensively studied.
The present contribution deals with the bioaugmentation of selected microorganisms in non-sterile soils contaminated by PAHs. In particular, an inoculum of 8 bacterial strains (*Arthrobacter protophormiae*, *Bacillus megaterium*, Psychrobacter sp., *Pseudomonas fluorescens*, *Pseudomonas veronii*, *Acinetobacter calcoaceticus*, *Pseudomonas putida* and *Pseudomonas stutzeri*) previously isolated from other contaminated sites was used. In order to avoid the costs of producing large amount of inocula and to concomitantly adapt bacteria to aromatic substrates, the consortium was inoculated in a real contaminated soil (7399 $mg.kg^{-1}$ of PAHs) and in an artificially contaminated soil (1540 $mg.kg^{-1}$ of PAHs) only at the first step. Hence, aliquots of each of the augmented soils served as inoculum for the subsequent step of dilution in their respective non-inoculated counterparts.
Total DNA was isolated from all soil samples, while the degradation of PAHs was monitored in each sample 4 months after the inoculation. During the second step of inoculation, the degradation of PAHs reached 70 and 80% of the original concentrations in the real contaminated soil in the case of the artificially contaminated soil, respectively. At first, the adaptation of the bacterial strains was evaluated using the DGGE method detecting 16S rDNA from the soil samples and from the pure bacterial cultures. Hence, due to low resolving power of the DGGE method with specific regard to Pseudomonads, 454-pyrosequencing was used to identify members of soil bacterial communities. Among the allochthonous bacteria, those showing significant adaptability were *Acinetobacter calcoaceticus* and *Pseudomonas putida* for the real contaminated soil and Acinetobacter calcoaceticus, *Arthrobacter protophormiae*, *Pseudomonas stutzeri* and *Pseudomonas putida* for the artificially contaminated soil.

Microbial activity of chromium polluted soil from Guanajuato México, during in situ biostimulation assay.

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Hexavalent chromium is a dangerous mutagen and oxidizing agent, highly soluble and able to enter bacterial and mammalian cells via sulfate transport systems. The excessive Cr(VI) industrial wastes and their improper disposal have resulted in an anthropogenic pollution of various environments including soils and aquifers. Diverse bacteria have developed several strategies to resist high concentrations of Cr(VI), mainly through chromate efflux and Cr(VI) reduction to Cr(III), which is highly insoluble, and less toxic and unable to permeate biological membranes. The microbial reduction of Cr(VI) can be enhanced by electron donor addition as a safe and cost-effective technology. This technology called biostimulation has been successful in several contaminated sites, however each one is a different challenge and the efficiency of different electron donors depends of indigenous microbial community and the physicochemical characteristics of each place. In previous studies in our lab, we test the effect of different electron donors in Cr(VI) contaminated soil and results showed that molasses was the best electron donor in batch experiments The present study was aimed to the evaluation of the efficiency of molasses as electron donor to promote the Cr(VI) reduction in situ by indigenous microorganisms of polluted soil from a deposit of industrial wastes located in Guanajuato, México. Samples of soil were taken at 20 and 40 centimeters of deep and Cr(VI) concentration and pH levels were quantify. Before biostimulation, samples showed a pH 8.5 and a Cr(VI) concentration of 5594 mg per kg of soil. After 15 days of biostimulation in situ, chromate was reduced 100% and the final pH up to 9. The soil assayed in batch studies presented a low microbial diversity with *Halomonas* as dominant genera. Metatranscriptomic analysis as well as complementary microbial diversity studies will be conducted to enhance the knowledge of metabolic activities of the indigenous microbial community involved in Cr(VI) reduction. The high efficiency of Cr(VI) reduction by biostimulation with molasses, suggest that this technology is a potential solution to the serious pollution problem on this site.

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Interactions of natural occurring eukaryotic microorganisms with uranium(VI)

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Despite high uranium concentrations (up to 14 mg/L) and low pH (2.5 – 3.0) a high microbial diversity was detected by culture independent methods in the flooding water of the former uranium mine Königstein (Saxony, Germany). In this study we used culture dependent techniques for the isolation of eukaryotic microorganisms from the flooding water. It was possible to isolate different eukaryotic fungi with a glucose rich medium. The microbial isolates identified by 16S rDNA and 18S rDNA were tested for their uranium tolerance abilities by the determination of the minimal inhibitory concentration (MIC) on solid media. The results showed high tolerances of uranium (up to 6 mM) on solid agar plates. Based on these results isolate KS5 (*Rhodosporidium toruloides*) and one reference organism DSM 10134 (*Rhodosporidium toruloides*) were selected for further uranium interaction experiments. Uranium biosorption tests indicated that the cells of the strain KS5 and the reference DSM 10134 are able to remove high amounts of uranium ranging between 120 and 160 mg uranium/g dry biomass. To test the uranium tolerance quantitative in liquid media flow cytometry experiments with KS5 and DSM 10134 were done. These results showed a higher uranium tolerance of the isolate KS5 compared to the reference culture of DSM 10134 like the tolerance test on solid medium. Summarizing the results of this study indicate that eukaryotic microorganisms within a uranium-contaminated environment could play an important role in the bioremediation of radionuclides.

Effects of compost addition and *Medicago sativa* occurrence on PCB biodegradation in a historically contaminated soil

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Polychlorinated biphenyls (PCBs) are organic hydrophobic persistent pollutants that were manufactured as commercial mixture of various PCB congeners. Their degradation occurs mainly by aerobic and anaerobic processes mediated by microorganisms. The microbial degradative activity can be promoted in soil by plant presence, because plant roots release exudates that can influence PCB biodegradation. In degraded soils for both presence of contaminants and low organic matter content, the choice of the plant species is essential for the selection of the microbial community, for the formation of a thick rhizosphere and for the soil nutrient enrichment, which can be further increased through the addition of compost. In order to investigate the interactions between plants and the autochthonous microbial community in the rhizosphere, soil samples, collected from a PCB historically contaminated area, were used to set up a degradation experiment in pots in the presence of *Medicago sativa*, as well as in the presence/absence of compost, derived from municipal solid waste, and treated with Apirolio. The experiment was performed for more than 220 days. Microbiological and chemical analysis were performed at different times in order to evaluate the structure and functioning of microbial populations in relation to the different experimental conditions and PCB degradation. The microbial community was studied in terms of abundance, viability, diversity and activity. The results highlighted that each treatment acted in a different way on transformation/degradation of the various PCB congeners. The Apirolio did not negatively affect the microbial community, but its addition stimulated the biodegradation activity and the decrease of those congeners which were added with it. In the hystorically contaminated soils the compost and plant favored both lower- and higher-chlorinated congeners degradation, even if acting at different times. In all conditions the compost and plant co-presence favored both microbial abundance and activity. The plant presence always favored an increase in Alpha-Proteobacteria and this result may be ascribable to the occurrence both of several nitrogen-fixing bacteria and PCB degrading bacteria belonging to this sub-class.

The possible application of microorganisms in promoting plant growth and improving plant biomass in the phytoremediation of anthropogenic and contaminated soils

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Understanding the correlation between rhizosphere microorganisms and plants is important in increasing plant growth in the process of supporting phytoremediation of heavy metal contaminated and anthropogenic soils.
This research focuses on the possible stimulatory effect of plant growth promoting rhizobacteria on plants biomass of *Festuca rubra* L. and *Brassica napus* L. cultivated on two different soil types (soil containing heavy metals and soil from the external spoil tip of the lignite mine). The rhizosphere microorganisms were collected from the roots of plants growing on contaminated area. Obtained isolates were tested for biochemical activity demonstrating the stimulatory effect. Bacterial isolates were also tested for in vitro growth inhibition of phytopathogenic fungi: *Alternaria* sp., *Fusarium oxysporum* and *Fusarium culmorum*, for possible application in the phytoremediation process, supported with sewage sludge. Based on the biochemical activity, pot testing was conducted. Plants were inoculated with the selected bacterial isolates.
The study confirmed that some rhizosphere bacteria has antifungal activity. It was also found that bacterial inoculation has stimulatory effect on the plants growth. Moreover, this effect occurs both, in relatively less contaminated mixture sandy soil and also in strongly contaminated soil. Inoculation with bacteria in sandy soil stimulated root growth and stem weight of *Festuca rubra* L. and stimulated the root growth of *Brassica napus* L. However, in metal contaminated soil, bacteria have a more pronounced impact on weight root and stem length of *Festuca rubra* L.
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Non-target effects of pesticides on the microbial activity in agricultural soil

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Short term effects of different pesticides (Carbofuran, Glyphosphate and Carbendazim) on microbial activity in terms of respiration and exo-enzyme production were examined in agricultural soil under laboratory conditions. Main factors examined included: 1) Pesticides (without, Carbofuran, Glyphosphate and Carbendazim), 2) Organic matter in terms of maize stover (0 and 0.5%) and 3) Phosphorus mineral fertilization (0 and 100 ppm P). Soil respiration was measured using the alkaline absorption method throughout the 23 days of incubation and enzyme activities in terms of glucosidase, fosfomonoresterase and N-acetyl-beta-D-glucosaminidase (NAG) were measured only at the end of the incubation.The results obtained showed that addition of the three pesticides increased the respiration of native soil microorganisms, indicating that the pesticides serve as energy and nutrient for the microbial community. Addition of organic matter also increased soil respiration as expected. In addition, the microbial respiration increased in treatments with both pesticides Carbofuran and Carbendazim when organic matter and P were added. Furthermore, the treatment with Glyphosate and Carbendazim, and P fertilization, but without organic matter had higher microbial respiration than the control samples. These results suggest that the P fertilization promotes soil microbial respiration. All factors examined also affected soil enzyme activities. Combination of Carbendazim + organic matter + P had the highest activity recorded for the three enzymes compared with the other treatments. In contrast, Glyphosate + organic matter + P and Carbofuran + organic matter reduced the activity of glucosidase and NAG. Regarding phosphomonoesterase, the treatments that included pesticides, organic matter and phosphorus showed the highest activity. In conclusion, the pesticides examined strongly affected soil microbial activity, but their impact seems to depend on the presence of both organic matter and mineral P in the soil, which should be considered when examining non-target effects of pesticides in soil ecosystems.

Factors influencing microbial community development during primary succession on spoil heaps after brown coal mining

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Post-mining spoil heaps built of the materials excavated from deep subsurface often contain only a little amount of available organic carbon and other macronutrients and have very low initial biological activity, which makes them an excellent model for studying primary succession. Spontaneous development of ecosystems on such barren substrates includes organic matter accumulation accompanied by successive changes in vegetation and microbial community composition. In this study, changes in the abundance of bacteria and fungi and in the composition of microbial communities during primary succession were investigated in a brown coal mine deposit area near Sokolov, Czech Republic, using phospholipid fatty acids analysis (PLFA). The study considered a chronosequence of sites undergoing spontaneous succession of 6, 12, 13, 21, 30, 45 and 54 years of age. During the succession, the biomass of the total microbial community measured as the total amount of PLFAs in soil samples increased approximately sixteenfold, from 11 nmol g^{-1} soil dry mass at the 6-year-old site to 179 nmol g^{-1} at the 21-year-old site and later decreased to 108 nmol g^{-1} at the 54-year-old site. Bacteria-specific PLFAs followed a similar trend, increasing from 5 nmol g^{-1} to 83 nmol g^{-1} and decreasing to 62 nmol g^{-1}. The increase of fungal biomass was slower, starting at an initial concentration of 0,6 nmol g^{-1}, reaching a peak of 22 nmol g^{-1} at 21 years and decreasing again to 6 nmol g^{-1} at 54 years. The 6-year-old site with no vegetation cover substantially differed from all the other sites, showing the greatest specificity of its microbial community. In addition to temporal succession, site-specific patterns were observed in microbial community development.

Sequential managed aquifer recharge leads to a high diverse microbial community resulting in a better attenuation of moderate degradable trace organic chemicals (TOrC)

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Managed aquifer recharge (MAR) systems (e.g., riverbank filtration, soil aquifer treatment) are natural treatment processes with low energy demand and little chemical input. In these systems, water infiltrates through the vadose and saturated zones where microorganisms play an important role for the removal of dissolved organic carbon (DOC) and trace organic chemicals (TOrC)1,2. Previous research demonstrated that under oxic conditions, a low content of biodegradable organic carbon as primary substrate resulted in a more diverse microbial community in MAR systems and an increase in the expression of genes related to TOrC biodegradation2. Based on these results the sequential managed aquifer recharge technology (SMART) was developed combining two infiltration steps with an in-between aeration step providing more favorable conditions for microbial degradation of TOrC in the second infiltration step. Further results by Alidina et al (2014) indicate that co-metabolic transformation of moderately biodegradable TOrC in MAR systems is most efficient in the presence of refractory organic carbon sources3,4.
In order to test this hypothesis we performed parallel lab-scale column experiments with two different water sources, Lake Tegel water from Berlin with a high content of refractory organic carbon (>4 mg/L) and Garching WWTP effluent consisting of mostly biodegradable DOC. Easy degradable DOC from both waters is removed during the SMART process in a first infiltration step, providing oxic, oligotrophic conditions with a significant difference of refractory organic carbon in the second infiltration system. The investigations of the composition and function of the microbial community receiving different primary carbon conditions was obtained by using shotgun and 16S rRNA metagenomics sequencing in combination with target analytical methods (LC-MS/MS).

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Ecological restoration of nickel mine sites in New Caledonia: Characterisation of ectomycorrhizal fungal community the key enabling the monitoring of facilitation process between plants

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Ecological restoration of nickel mining sites operated as open pit in New Caledonia, a Hot Spot of biodiversity, remains challenging. To meet this challenge, in a project jointly developed by Koniambo Nickel SAS, CIRAD and IAC, we explored the use of the triple symbiotic *Acacia spirorbis* as nurse plant to enable the re-implantation of target endemic species and thus facilitate the further sustainable re-establishment of a diversified and endemic ecosystem through natural plant succession.
Taking into account the importance of mycorrhizas for facilitation between plants, and that, whatever the soil *A. spirorbis*, the nurse plant, is found to be associated with a variable range of ectomycorrhizal fungi composed of about 140 OTU; the same being true for *Tristaniopsis* spp., the main target genus, also found associated with a variable range of ectomycorrhizal fungi. The composition of ectomycorrhizal communities spontaneously established with *Acacia spirorbis* and *Tristaniopsis* spp. has been described using molecular characterization of ITS in different plantations (*A. spirorbis*) and natural stands (*A. spirorbis* and *Tristaniopsis* spp.) on the Koniambo massif. New plantations of *A. spirorbis* have also been established in year 2014; the evolution of their ectomycorrhizal communities since eighteen months is here reported.
The comparison of ectomycorrhizal communities between *A. spirorbis*, the nurse plant and *Tristaniopsis*, the target genus enable us to consider *Acacia spirorbis* as a good candidate to play the role of nurse plant for the further sustainable implantation of *Tristaniopsis* spp.
These results enable the further development of field trials aiming at the setting of an assisted plant succession. The ultimate goal of this assisted plant succession is the eradication of the nurse plant.

Boreal acid sulphate soils – changes in bacterial communities along vertical profile and between total and active pools

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Acid sulphate (AS) soils located along the Baltic Sea coast contain large carbon, nitrogen and sulphur stocks in subsoils. If AS soils are drained and used for agriculture, large fluxes of carbon and nitrogen gases could therefore occur. We aimed to compare bacterial community composition of AS soils in 4 soil horizons in comparison to non-AS soil (N-AS) assessed by 16S-pyrosequencing. We sampled three AS soils – Patoniitty (P), Söderfjärden (S), and Ylistaro (Y) with the different cultivation history, and one N-AS, Alaniitty (A). A and P soils are located in close vicinity. Four different soil horizons were studied: Ap, Bg, BC, and C horizons. AS and N-AS soils differed in the amount of organic C, total N, sulphur species, as well as in pH and microbial activities in the C horizons. In topsoils, RNA-based bacterial community was studied too. Interestingly, we observed relatively high bacterial diversity together with high microbial acitivities and biomass in deep subsoils. Main drivers of bacterial community composition were pH and redox potential. Bacterial communities in Ap horizons were dominated by Acidobacteria, Proteobacteria, Planctomycetes, Gemmatimonadetes, and Chloroflexi, while in subsoils, Proteobacteria increased at the expense of Acidobacteria. The differences between AS and N-AS soils increased with soil depth, being characterized by the enrichment of Gemmatimonadetes and Chloroflexi in N-AS soil; and Actinobacteria, Spirochaetes and Cyanobacteria in AS soils. The highest shifts has been observed in C horizons, where the higher abundance of Candidate division OP9, Planctomycetes, and Chloroflexi has been observed in AS soils in comparison to N-AS. In C horizons of AS soils, sulphate reducing bacteria were enriched, represented by Desulfarculales, Desulfobacterales, Sva0485, and Syntrophobacterales. Topsoil Acidobacteria (Ac) communities did not differ, while in subsoils, different Ac communities dominated in AS soils (Ac group 2) in comparison to N-AS soil (Ac group 6). Active Ac community was represented by Ac group 3, while in total one Ac group 6 dominated. RNA-based bacterial communities of topsoils were dominated by genera: *Pirerulla*, *Gemmata*, *Planctomyces*, *Caldilinea*, *Phaselicystis*, Candidatus *Solibacter*, *Caenimonas*, and *Haliangium*. To conclude, boreal AS soils in deep subsoils harbour diverse and active bacterial communities putatively associated with anaerobic methane oxidation coupled with sulphate reduction.

Bioremediation of chlorendic acid, a highly chlorinated organic pollutant, by exploiting a fungal-bacteria consortium native to the contaminated field

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Bacteria as well as fungi are known to be able to degrade organic pollutants. Bacteria can use organic pollutants as a carbon source and metabolize them. Fungi can produce extracellular enzymes that act on a broad array of organic compounds, thereby degrading them. In this study, the possibility to remediate an industrial site contaminated with the highly chlorinated organic pollutant, chlorendic acid, a fire retardant and probable carcinogen, by exploiting bacteria and fungi native to the site is investigated. Plants may enhance bioremediation by promoting the growth and activity of contaminant-detoxifying microorganisms in soil. Moreover trees with a high transpiration rate, like poplar, draw in high amounts of contaminated groundwater and bring it into close contact with the degrading microorganisms. The ultimate goal of this research is therefore to isolate chlorendic acid-degrading microorganisms and to test their bioremediation efficiency in combination with poplar.
To find suitable microorganisms, bacteria and fungi were isolated from soil, rhizophere and roots of poplar and grasses growing on a chlorendic acid-contaminated site. Of the 75 isolated fungal strains, 4 significantly lowered the concentration of chlorendic acid after 2 weeks, one even to 29% of the original concentration. These degrading fungi were further investigated for laccase and peroxidase activity as these enzymes can be responsible for the degradation of recalcitrant compounds. After selective enrichment of a soil sample, 1 bacterial consortium was found to significantly decrease the concentration of chlorendic acid, but only to 94% of its original concentration. The role of the isolated fungi in the degradation of chlorendic acid therefore seems more important than that of the bacteria. However, the isolated bacteria can still be important because they can promote plant growth. Therefore they were screened for different plant growth-promoting traits, after which a consortium was selected, consisting of chlorendic acid-degrading fungi and plant growth-promoting bacteria. The effect of inoculation of poplar with this consortium on the bioremediation efficiency is currently being evaluated in a greenhouse experiment.

Omics approach in analysis of *Pseudomonas mandelii* ssp. capable of bioaccumulating hexachlorocyclohexane

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Background and Aims
Knowledge about majority of soil microorganisms and their metabolism of toxic compounds still remains elusive. As prokaryotes are known for their high adaptability to different environmental conditions, there is very likely that soil bacteria poses ability for production of vast number of bioactive molecules involved in pesticide decomposition.
Our aim was to identify microorganisms capable of degrading hexachlorocyclohexane (?HCH) and investigate this metabolic process by bioinformatic analysis of deeply sequenced bacterial genome coupled with transcriptomic data from RNA-seq and proteomic data from mass spectrometry.
Methods
Bacteria were isolated from heavily contaminated soil samples, derived from expired pesticide storage infrastructure in Poland. Isolated microorganisms were tested for growth abilities on media with pesticide as a main carbon source.
Genomic DNA and mRNA were isolated from pure cultures and sequenced using Illumina HiSeq and MiSeq platforms. Genome was assembled using SPAdes software. Transcriptomic and proteomic profiles were compared for bacteria living on a medium with and without ?HCH.
Results
Screening of isolated bacteria resulted in identification of *Pseudomonas mandelii* ssp. exhibiting ability to form a clear zone around the colony, what indicated its capability for ?HCH degradation. Reads assembly form genome sequencing resulted in nearly complete 6Mb genome. Intriguingly, analysis of assembled genome did not revealed presence of known genes coding proteins involved in ?HCH degradation. Differential RNA-seq analysis revealed that mRNA coding 5 specific proteins is elevated 25 to 60 times. Further studies showed that four of identified genes are localized in the same locus possibly creating an operon. Most of them encode proteins from the family of quinohemoprotein amine dehydrogenases. Other elevated transcripts corresponded to membrane transport proteins and antioxidant enzymes, suggesting induction of oxidative stress in cell. On the other hand, shotgun proteomics analysis showed a great increase in number of proteins from central metabolic pathways and lipid biosynthesis but also stress-related proteins. Further investigations will be conducted to confirm the significance of obtained results in ?HCH response.
Keywords: Pesticide; Hexachlorocyclohexane; Genomics; Transcriptomics

Diversity of bacteria involved in ^{13}C-labelled wheat root decomposition and efflux-mediated metal resistance in metal contaminated soils remediated with amendments

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Agriculturally used soils in the Mashavera Valley in southeast Georgia are mostly contaminated with trace metals (Cd, Cu, and Zn). Due to the large scale of the site, conventional remediation technologies are insufficient or too expensive. An alternative is an in situ immobilization of the metals by amendments. In 2010 Hanauer et al. (2012) conducted a pot experiment to test the amendments iron grit, natural zeolite, and Divergan® to decrease the mobile metal fraction in the soil solution. The soil used for the experiment was a topsoil of a Kastanozem (0–20 cm) of an arable field situated close to Bolnisi, and characterized by a neutral pH, high content of clay and organic carbon. After 12 months mobile metal contents amounted to 0.24 mg kg^{-1} Cd, 1.59 mg kg^{-1} Cu, and 18.41 mg kg^{-1} Zn in the control, to 0.07 mg kg^{-1} Cd, 0.95 mg kg^{-1} Cu, and 4.15 mg kg^{-1} Zn in the iron grit treatment, to 0.06 mg kg^{-1} Cd, 1.54 mg kg^{-1} Cu, and 1.20 mg kg^{-1} Zn in the zeolite treatment, and to 0.00 mg kg^{-1} Cd, 0.52 mg kg^{-1} Cu, and 0.03 mg kg^{-1} Zn in the Divergan® treatment.
Alterations in the composition of microbial communities have been proposed to be a sensitive indicator of trace metal effects. Culture-independent community structure comparisons in short- and long-term metal contaminated soils have shown differences in the microbial diversity (Kaplan et al. 2014). The remediation may stimulate the recovery of the microbial diversity in metal contaminated soils. Fife years after remediation of the soil metal concentrations were analyzed again to assess the stability of remediation. We aim to identify cellulolytic bacteria as one important functional group in soil using ^{13}C-labelled wheat roots for stable isotope probing. Furthermore the occurrence of efflux-mediated metal resistance genes for Cd, Cu, and Zn in the remediated soils was analyzed by real-time PCR with specific primer pairs.

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Bioinformatic approach to analysis of plasmid pool in metagenomes from polluted soils

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Plasmids are bacterial mobile genetic elements that facilitates rapid evolution and adaptation of their hosts to changing environmental conditions. Soil is the environment inhabited with thousands of species of bacteria and animals, having a large impact on its structural and chemical properties and, as the result, on its fertility. As genes coded on plasmids has a big impact on their bacterial hosts, their importance for soil properties and fertility cannot be disregarded.

There is many reports of plasmid diversity in soil, however, they are usually based on PCR amplification of fragments specific to given classification group, or isolation of single plasmids. Although several reports introduce metamobilomics approach, they are limited to simple environments and rather small plasmids.

The objective of this work was to characterize plasmids present in the organochlorine pesticide contaminated soils coming from expired pesticide burial tombs using novel bioinformatic approach.

First, sequenced bacterial and Archaeal genomes, as well as all known plasmids, where downloaded from NCBI. Sequences were processed to obtain kmer frequency profiles and obtained profiles were used to train statistical model used to discriminate between sequences of chromosomal or plasmid origin. Model was validated using publicly accessible metamobilomic data.

Next, model was applied to metagenomic contig sequences obtained from soil samples varying in the pollution level. This approach allowed us to retrieve many contigs of probable plasmid origin. Obtained sequences where annotated using Blast to confirm presence of plasmid structural genes. Analysis of obtained annotation profiles revealed presence of many organochlorine degradation and resistance related genes.

Obtained results indicate that model developed for idetnification of plasmid sequences can be succesfully applied to environmental samples with high biodiversity.

Ecology of soil bacteria in bioremediation: indigenous plant growth promoting rhizobacteria in native *Spartina maritima* as a tool for the restoration of heavy metal polluted salt marshes

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The joint estuary of Tinto and Odiel rivers in Spain is one of the most polluted areas by heavy metals in the world. It drains the region of the world's oldest continuously operating mine and the anthropogenic intervention is represented by the strong industrialisation of the area. In these estuaries, the indigenous cordgrass *Spartina maritima* grows naturally, making it useful for phytostabilization of estuarine sediments. This natural area is protected by provincial and state policy plans and needs restorative intervention urgently.
Cultivable bacteria were isolated from the rhizosphere of *S. maritima* in polluted estuaries and characterized. The best-performing strains were selected attending to metal resistance and plant growth promoting (PGP) traits. Their efficiency was tested in greenhouse inoculation experiments with natural plants and soils. Concurrently, Denaturing Gradient Gel Electrophoresis (DGGE) was performed for rhizosphere and bulk soil in contaminated and non-contaminated estuaries in every season throughout an entire year.
DGGE results, influential for land decisions, indicated that microbial diversity in these soils is not completely related to the plant species they inhabit with, but to environmental conditions. Non-polluted estuaries showed the highest bacterial biodiversity, deeply affected by seasonal changes, whereas in polluted soils either plant roots nor seasonal variation had any marked effect on the bacterial populations observed. Regarding inoculation experiment, bacterial consortium significantly enhanced the efficiency of metal phytostabilization in natural soils. Inoculated *S. maritima* plants increased their belowground biomass and showed improved physiological effects. Besides, plant metal uptake in roots was stimulated, increasing up to 50% for Cu. Currently, a transcriptomic approach on these effects is being developed. In the near future, Andalusian authorities may approve an in situ project to test the utility of this consortium in vivo, as planned within regional projects agenda.
On the basis of these results, the inoculation of *S. maritima* with indigenous metal-resistant PGP rhizobacteria may be used as an efficient method to increase plant adaptation and growth during restoration experiments. On the whole, this work is an important approach about the utmost important role of native soil bacterial communities in conducting restoration strategies in polluted scenarios in order to preserve native ecosystems.

Interactions of nano zerovalent iron with *Acidithiobacillus ferooxidans* – Implications for soil remediation

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Nano zero valet iron (nZVI) presents perspective agent usable for remediation of soils and groundwater contaminated with wide variety of both organic and inorganic contaminants. After application to soil, nZVI particles readily oxidize and transform to Fe nanooxides. These particles possess considerable sorption properties being thus potentially efficient agents for immobilization of metals/metalloids in contaminated soils. *Acidithiobacillus ferooxidans* is a bacterial strain used in bioleaching technologies applicable for biomining, metal waste treatment and environmental remediation. The aim of the study was to examine the influence of nZVI on the mobility of metals in contaminated soils and its interactions with Fe-oxidizing bacteria *Acidithiobacillus ferooxidans*. After 30 days of incubation in soil highly contaminated with Pb, Zn and Cd, nZVI (1% w/w) application was able to decrease the amount of Pb, Zn and Cd in soil solution by 50%, 80% and 80%, respectively. Addition of nZVI (0.1%; v/v) to cultivation medium has positive influence on the bacterial culture growth. When the bacteria were added both to control soil and soil previously treated with nZVI, they considerably increased the leachability of main contaminants in soil during first 2 weeks. However, the leachability of these metals rapidly decreased after 4 weeks reaching lower values than those recorded for control. These preliminary results show the need for further investigation of processes involved in dynamic bioleaching process.

Oil-degrading bacteria isolated from the rhizosphere of plants growing in oil-contaminated soils from Kazakhstan

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Quantitatively, oil and oil products are the main environmental pollutants in the world. Rhizoremediation, which can be used at the final stages of polluted soil re-cultivation, is based on the use of microorganisms to utilize environmental pollutants as carbon and energy source, and leads to the destruction of organic pollutants by microorganisms associated with plant roots. Therefore microbial strains were isolated from the rhizosphere of alfalfa (*Medicago sativa*), grass mixture (*Festuca rubra* – 75%, Lolium perenne – 20%, *Poa pratensis* – 10%) and rape (*Brassica napus*) on the basis of their high capacity to use crude oil as the sole carbon and energy source. These isolates used an unusually wide spectrum of hydrocarbons as substrates (more than 80), including n-alkanes with chain lengths ranging from C12 to C32, monomethyl- and monoethyl-substituted alkanes (C12 – C23), n-alkylcyclo alkanes with alkyl chain lengths from 4 to 18 carbon atoms as well as substituted monoaromatic and diaromatic hydrocarbons. These three strains were identified as *Gordonia rubripertincta* SBUG 1971, *G. rubripertincta* SBUG 1972 and *Rhodococcus* sp. SBUG 1968. During their transformation of this wide range of hydrocarbon substrates a very large number of aliphatic, alicyclic and aromatic acids was detected, 44 of them were identified by GC/MS analyses and 4 of them are described as metabolites for the first time. On the basis of all transformation results we conclude that the main components of the Kazakh crude oil can be degraded by all three strains. Inoculation of plant seeds with these highly potent bacteria had a beneficial effect on shoot and root development of plants grown on oil-contaminated sand. For rhizoremediation an important contribution to the degradation of pollutants is attributed to microorganisms suggesting that the newly isolated species might be used for rhizoremediation projects in Kazakhstan and elsewhere.

Pyrosequencing reveals bioaugmentation impact on the dynamics of bacterial community on phenanthrene-contaminated soil

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Bioaugmentation, the adding of allochthonous degrading bacteria or consortium, is a complex technique because its effectivity depends on the interaction between the inocula and the indigenous population. The impact of the inoculation of a phenanthrene-degrading consortium (CON) and a *Sphingobium* strain (AM), isolated from the CON, on soil bacterial community dynamics was evaluated on phenanthrene-contaminated soil. The shift produced by AM on the bacterial community of unpolluted soil was also determined. The bioaugmentation studies were performed in soil microcosms contaminated with 2 g of phenanthrene/Kg dry soil. The microcosms were inoculated with 1.4x108 cfu/g dry soil, and incubated for 63 days. An unpolluted soil microcosm was inoculated with the strain AM. The respective non-inoculated microcosms were used as control. The phenanthrene concentration (HPLC) was determined at different times and 16S rRNA gene pyrosequencing analysis was performed at 14 and 63 days of treatment. After 63 days of incubation, the phenanthrene concentration in non-inoculated microcosm was 73 mg/Kg dry soil; the inoculated microcosms with CON and AM showed phenanthrene concentrations significantly lower, 24 and 6 mg/Kg dry soil respectively. Pyrosequencing generated a mean of 4743 quality-filtered reads per sample that were grouped into 2228 OTUs using 97% similarity. According rarefaction curves the number of sequences was enough to cover most of the diversity. The Hill-numbers showed a reduction in bacterial richness (0D) and diversity (1D, 2D) in all contaminated microcosms. This effect was not noticed in the inoculated unpolluted soil microcosm. After 63 days of treatment, only the inoculated microcosms recovered 0D values similar to the control, but with a reduced diversity. The contamination with phenanthrene produced a stimulatory effect on the orders Actinomycetales, Burkholderiales and Sphingomonadales. Whereas the predominance of Actinomycetales and Sphingomonadales increase after 63 days of treatments, the Burkholderiales rapidly decline. Both inoculums produced an additional increase of Burkholderiales and Sphingomonadales, with a relative decline of Actinomycetales. While the establishment of the strain AM depended on the PAH presence, the inoculation with this strain produced an early increase of Rhizobiales, independently of the phenanthrene presence. Both inoculums stimulated the phenanthrene degradation and managed to recover the soil bacterial richness.

Effect of olive and vine wood ashes on the dehydrogenase activity in a crop land

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Wood ashes resulting from burning olive marc and vine pruning waste for electric power production are starting to accumulate and therefore alternatives are demanded. The mineral composition of the ashes suggests that they can be used not only to improve the physical and chemical features of acid and degraded soils but also as fertilizers in crops areas as agents capable of increase the nutritional capabilities of the crop land. Nevertheless, the main problem using ashes is the impact that the high pH might have at medium and long term, which could affect negatively the whole environment. In the framework of the evaluation of the use of wood ashes to be applied in farmland, we try to improve the soil nutritional capabilities, the reintroduction of ashes in a sustainable way and to avoid the negative impact that the high pH may have. For this purpose, we investigated the effect that ashes may have on the soil microbial activity. Dehydrogenase activity (DHA) was measured in laboratory as a function of ash application rate and type (olive and vine). Our hypothesis is that different wood ashes, and different proportions of them, may affect the microbial activity in different ways and could have beneficial or detrimental effects depending on the applied rate. The results show that the DHA is very sensitive to the presence of ashes. The exponential fitted line drops strongly when the ashes concentration increase. Although the mineral composition and pH of both ashes are different, the fitted curves show a similar behaviour. After comparing the DHA with the electric conductivity data obtained by the different ash concentration treatments, it can be seen that the negative effect of ashes on the DHA is not only linked to the change of electric conductivity but also to the particular ash composition.

Bacterial community characteristics under decades-lasting antibiotics selection pressures

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Presented study is the first metagenomic characterization of the bacterial consortia of soils that have been exposed to long-term selection pressure of β-lactam pollutants at manufacturing sites.
The study of the community changes brought about by β-lactam antibiotic presence can provide an early warning system for anticipation of bacterial resistance development and potentially assist in the development of next-generation antibiotics against resistant strains. Moreover, a deeper understanding of the microbial diversity that exists below-ground can help to explain the ecological mechanisms, to develop conceptual models of the factors controlling microbial diversity and the ability to understand and predict distribution patterns.
To investigate the changes in bacterial community characteristics under long-term antibiotic selection pressures, soil samples from the antibiotic-manufacturing plant in Central Europe (continuous production of penicillin G, V, and semi-synthetic antibiotics since 1956), were taken and analyzed.
Illumina sequencing of V4 variable region of 16S ribosomal RNA gene revealed very complex bacterial communities. Principal component analysis, used to identify the differences in community structures, has shown that the two types of communities (polluted and reference) could be well separated at the genus level and with lower resolution also at phylum level.
Contrary to the assumptions, the antibiotic presence in soil did not reduce the diversity of bacterial communities in polluted soil samples compared to those in the two reference samples. Moreover, microbial diversity expressed by Shannon diversity index H was significantly higher in the polluted soil and increased mainly by the species contributing to less than 0.5% of the overall sample composition.
Ratios/percentages of antibiotic resistant strains in bacterial communities in soil samples were estimated by a pilot culture-based analysis. Obtained data showed that the overall resistome of the polluted soil communities was boosted not only in terms of resistance to β-lactam antibiotics but also against the tetracycline, macrolide, chloramphenicol, or aminocyclitol class of antibiotics.
The study demonstrates a significant impact of anthropogenic activity on soil microbial consortia and justifies the need for additional analyses to fully understand the effect on the composition, ecology, and especially the resistance in the soil bacterial communities of adjacent area.

Response of soil microbial community to titanium dioxide nanoparticles: a cascading pitch on the nitrogen cycle

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Soils are facing new environmental stressors, such as titanium dioxide nanoparticles (TiO_2-NPs). While these emerging pollutants are increasingly released in most ecosystems, including agricultural fields,, their potential impacts on soil and its functioning remains to be investigated. Here we report the response of the microbial component of an agricultural soil exposed over 90 days to TiO_2-NPs (1 and 500 mg kg^{-1} dry soil). To assess their impacts on soil functioning, we focused on the nitrogen cycle and thus measured nitrification and denitrification enzymatic activities combined to the quantification of their representative genes (*amoA* for ammonia-oxidizers, *nirK* and *nirS* for denitrifiers), as well as to the bacterial, archaeal and ammonia-oxidizers diversity changes. With strong negative impacts on nitrification enzymatic activities and on the abundances of ammonia-oxidizing microbes, TiO_2-NPs triggered a cascading negative effects on denitrification activities and a deep modification of bacterial community structure, even after 90 days exposure at low, but realistic NPs concentrations. We encourage further research to assess how these modern pollutants modify soil ecosystem functioning and the related soil fertility

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Composition and activity of microbial communities in soil contaminated by heavy metals

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The poster focuses on studying changes of microbial communities living in the soil contaminated by heavy metals. Two sites with different degree of contamination were selected in the Příbram area. Respiration was measured in vitro in the soil samples supplemented with various carbon sources and different concentration of cadmium. The respiration showed that even at cadmium concentration of 1000 $mg.kg^{-1}$ the community is viable and capable of utilization of substrates while increasing the respiration rate.
Enviromental DNA from soil samples was isolated and 16S rRNA gene of actinobacteria was amplified. The terminal restriction fragment length polymorphism analysis showed a clear difference between the profiles of both sites. The shifts in the community profiles were observed also after the addition of substrates.
The quantification of total bacteria and actinobacteria was performed by quantitative PCR based on amplification of part of the 16S rRNA gene. The more contaminated site contained slightly more bacteria, but almost twice the actinobacteria than the less contaminated one.
The conclusion was made that, high long-term contamination with heavy metals increases respiratory activity even more so under stress conditions induces by added cadmium and reduced stability of the bacterial community.

Influence of sources of carbon in growth media on the yield of biosurfactant by the microbe isolated from crude oil contaminated soil

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Biosurfactant producing bacterial strains were isolated from crude oil contaminated soil samples. Isolation was carried out by adding 1 g of collected soil sample to the mineral medium containing crude oil (2% w/v) as the sole carbon source. The *Pseudomonas aeruginosa* RS29 strain was isolated from the soil and tested for its efficiency in biosurfactant production. In the present investigation, RS29 was tested for its ability to produce biosurfactant on various water miscible and immiscible carbon sources.
Biosurfactant production was estimated in terms of surface tension reduction and emulsification index (E24). Surface tension was measured in a K11 tensiometer (Kruss, Hamburg, Germany). The carbon sources used in the investigation were glucose, glycerol, mannitol, n-hexadecane and olive oil.
The isolated strain showed better production of biosurfactant on water soluble carbon sources than water insoluble ones. Glycerol was found to be the best one for maximum biosurfactant production. Maximum surface tension reduction was achieved (50.46%) in glycerol with E24 of 72%. Biosurfactant production was positively correlated with the growth of *P. aeruginosa* RS29. Maximum surface tension reduction was obtained (27.0 mN/m) after 44 h of growth. The biosurfactant was produced as a primary metabolite.

Abandoned tropical tin mine site shows changes in microbial community with restoration

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A tremendous number of mine sites have been abandoned in recent years, necessitating restoration efforts. Most mine restoration projects in most climates have included tree plantation as a basic technique, as so most of the efforts has been put on the monitoring of the planted trees. However, since microbes are sensitive to environmental changes and play a major role in nutrient cycling, this has strong potential as a useful measure of the progress of restoration.
In this study, we assessed edaphic conditions and microbial community composition of an abandoned tin mine site in Bidor, Malaysia which, has been undergoing restoration efforts for over 15 years. Samples were collected at three different sites: 1) the unrestored area of mine tailings 2) a site restored with *Acacia* species 3) the other from 3 different tropical forest sites in Malaysia as a control. Soil DNA was extracted and PCR amplified targeting 16S rRNA gene for bacteria, and ITS1 region for fungi followed by Illumina MiSeq sequencing. In an ordination, bacterial and fungal community of the three sites were significantly different with each other and the *Acacia* planted site was placed in the middle. At the phylum level, the relative abundance of Proteobacteria was significantly lower in the unrestored area compared to the other two sites. In the case of fungi, the relative abundance of Ascomycota decreased with restoration. The relative abundance of Basidiomycota was about five times greater in the normal rainforest compared to the other two sites.
These combined results suggest that the restoration effort has had a degree of success shifting the site conditions towards those of normal tropical rainforest. However, further efforts or more time for ecosystem development will be needed if the restored system is to closely resemble tropical rainforest.

Abundance and diversity of ammonia oxidizing archaea and bacteria in long-term industrial effluent polluted soils, Gujarat, Western India

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Ammonia oxidation, the first and rate limiting step of nitrification, is catalyzed by both ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). Recently, the role played by soil nitrifiers has gained much interest in monitoring the soil disturbances caused by a variety of contaminants. In the present study, abundance and diversity of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were investigated in long-term industrial waste effluent (IWE) polluted soils. Three different IWE polluted soils characterized as uncontaminated (R1), moderately contaminated (R2) and highly contaminated (R3) were collected along Mahi River basin, Gujarat, Western India. Quantitative numbers of ammonia monooxygenase α-subunit (*amoA*) genes as well as 16S rRNA genes indicated apparent deleterious effect of IWE on abundance of soil AOA, AOB, eubacteria and archaeal populations. Relatively, AOB abundance was found to be more than AOA in the highly contaminated soil R3, while predominance of AOA was noticed in uncontaminated (R1) and moderately contaminated (R2) soils. Soil potential nitrification rate (PNR) significantly ($P < 0.05$) decreased in polluted soils. Denaturing gradient gel electrophoresis (DGGE) analysis of functional *amoA* genes revealed pollution induced community shifts in response to the degree of IWE contamination. Reduced diversity accompanied by apparent community shifts of both AOB and AOA populations was detected in R2 and R3 soils. AOB were dominated with Nitrosospira-like sequences, whereas AOA were dominated by Thaumarchaeal “group 1.1b (Nitrososphaera clusters). We suggest that the significant reduction in abundance and diversity AOA and AOB could serve as relevant bioindicators for soil quality monitoring of polluted sites.

Composition of fungal and bacterial communities in mercury polluted areas

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Mercury is highly toxic element belonging to the group of heavy metals, which occur in environment either naturally (e.g. geogenic - in form of HgS; due to wildfire emissions) or anthropogenic, due to the deposition from combustion of fossil-fuels, ore mining or industrial activity. Currently, human activities result in mercury emission of ca. 2000 metric tons per year. Nowadays mercury pollution in many places poses a serious problem. In our survey we have studied soil microbial communities on 4 mercury polluted sites in the Czech Republic. Hg content ranging ca. from units to hundreds of ppm. Microbial communities composition and relative abundances of specific fungal and bacterial taxas are significantly differ on the base of mercury content. More then 80 mercury-resistant bacterial strains were isolated on threshold of 0,1mM Hg concentration in medium.

Molecular characterization of the rhizobacterial communities of two Ni-hiperaccumulating subspecies of *Alyssum serpyllifolium* endemic to the Iberian Peninsula

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Serpentine soils, derived from ultramafic rocks, are characterised by low nutrient and organic matter contents, low Ca/Mg ratios, and high concentrations of trace elements. These soils constitute a hostile environment for plant growth and often support a unique flora with particular adaptations, such as mechanisms for (hyper)accumulation of trace elements (such as Ni). Hyperaccumulating plants are of great interest for phytoextraction or phytomining techniques. The study of the rhizobacterial communities of hyperaccumulating species is particularly interesting because of their potential role in the hyperaccumulation process. In this study the bacterial communities in the rhizosphere of two Ni hyperaccumulating subspecies of *Alyssum serpyllifolium*, endemic to serpentinitic areas in the NW (Melide, subsp. *lusitanicum*) and S (Sierra Bermeja, subsp. *malacitanum*) of Spain were characterised. These rhizobacterial communities were compared with the communities present in non-vegetated soil collected in the same serpentine areas.
The study included the analysis of 16S rDNA fragments by DGGE and 454-pyrosequencing, as well as the quantification by qPCR of the abundance of the gene *nccA* involved in bacterial resistance to Ni, Cd and Co.
In general, based on the pyrosequencing data and the DGGE profiles of the bacterial communities a clear separation between the two *Alyssum* rhizospheres and two bulk soils was found. Statistical analysis indicated that the similarity between the communities present in the two non-vegetated soils tended to be higher than that found between the two rhizobacterial communities. The pyrosequencing libraries contained OTUs characteristic of, or more abundant in, the rhizosphere soil than the non-vegetated soil. Thus OTUs highly similar to sequences from *Herbaspirillum* sp. and *Desulfonatronum* sp. from phylum Proteobacteria or *Flavisolibacter* sp. from phylum Bacteroidetes and *Lentzea* sp. from phylum Actinobacteria were mostly detected in rhizosphere soil. Some other OTUs were predominantly present in the serpentine areas from the NW or the S of the Iberian Peninsula.
The abundance of the *nccA* gene was higher in the rhizosphere soils of the Ni hyperaccumulating plants than in non-vegetated soils. Moreover, the *nccA* copy number in non-vegetated soil was significantly higher in Sierra Bermeja than in Melide.

Biotic and abiotic factors affect the colonization and the dynamics of bacterial community assemblage in irradiated soil microcosms

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Ecosystem dynamics are strongly related to community composition and diversity, factors that determine how communities respond to changes in the environment. Soil bacterial communities are known to harbour high levels of diversity, explaining their resilience to environmental disturbances. Secondly, colonization of pristine substrates, the product of ecological drift, is recognized as a stochastic process, but very little is known about how long it takes a community to colonize a pristine substrate and achieve a high degree of stability. Therefore, we sought to (1) study changes in the bacterial community structure during colonization (the very early stages of primary succession), and (2) evaluate resistance and resilience of communities to pulse type disturbances at the beginning of colonization. To test pulse disturbances we use cycloheximide (CHX), which is used for the exclusion of eucariota predators of bacteria (biotic disturbance), and 2,4-dichlorophenoxyacetic acid (2,4-D), which is an herbicide (abiotic disturbance). Soil from an agricultural plot was subjected to irradiation and then inoculated with non-irradiated (native) soil in a 1:19 ratio (native: irradiated). The experimental design included three treatments: (C) control (1 native: 10 irradiated), (B) biotic (control + CHX) and (A) abiotic (control + 2,4-D). Two replicates per sample were collected during 11 sampling times (0 to 300 h) for each treatment. Metagenomic DNA was extracted from each sample. T-RFLP with two restriction enzymes was used to bacterial community profiling using the 16S rRNA gene, as a phylogenetic marker. For each dataset, exploratory analysis was performed and ecologic networks were inferred using the Lotka-Volterra model. The results demonstrated that bacterial communities change drastically during the first 24 h of colonization and exhibit a high degree of resilience to the disturbances introduced. Furthermore, the degree of resilience was related to the disturbance type. Concomitant with the resilience indexes, the rate of change derived from ecological indexes fluctuated considerably during the early stage of colonization (from 1 h to 24 h), but stabilized over time. The decreased rate of change in the bacterial community structure may be used as an indicator of overall community stability. We concluded that soil bacterial community assemblage is a fast and dynamic process, characterized by minimal changes in community dynamics once stability is achieved.

Metal tolerance and biosorption potential of endophytic fungi isolated from *Bahia absinthifolia*

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The aim of this study was to select native microorganism able to remove and recover heavy metals in aqueous systems. Metal-resistant endophytic fungi isolated from the root of *Bahia absinthifolia* grown in soil contaminated in San Luis Potosí, Mexico was identified as *Macrophomina phaseolina*. Tolerance index and minimum inhibitory concentrations (MIC) for arsnic, copper, lead and zinc were determined. MIC for As^{5+} 15 and 7.5 mM, Zn^{2+} 12.5 and 10 mM, Pb^{2+} 7 and 5 mM and Cu^{2+} 5 and 1 mM in PDA and MES buffered minimal medium (MBMM) respectively. *M. phaseolina* was tested for their metal biosorption potential for As^{5+}, Cu^{2+}, Pb^{2+} and Zn^{2+} in vitro. Biosorption experiments were conducted with initial metal concentrations of 1 to 4 mM for As^{5+}, 0.5 mM for Cu^{2+}, 1 to 3 mM for Pb^{2+} and 1.5-10 mM for Zn^{2+} with a contact time of 4 h and wet fungal biomass (1–5 g) at 25°C. Maximum biosorption was found at 1 mM for As^{5+}, 0.5 mM for Cu^{2+} and 1.5 for Pb^{2+} and Zn^{2+} of initial metal concentration at the first hour. *M. phaseolina* accumulated 8.4 mg of As^{5+}, 1.8 mg of Cu^{2+}, 12.1 mg of Pb^{2+} and 16.9 mg of Zn^{2+} per gram of biomass. The effect of heavy metal on morphology was observed by scanning electron microscopy (SEM). The morphology changed in presence of heavy metal except with arsenic. Accumulation of these four metals by *M. phaseolina* indicated promising biosorption of arsenic and zinc and less of copper and lead from aqueous solution. This study increases the number of microbial strains as potentially low-cost biosorbents and can be an interesting alternative for bioremediation technology to remove and recover heavy metal ions from an aqueous solution.

Persisting in slag – insights into aluminium resistance from early industrial mineral leaching

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The leaching of sulfur-containing minerals such as alum from soft coal for industry was common until the end of the 19th century. Significant amounts of leached slag as by-product were dumped into the environment creating anthropogenic environments that still shape the landscape.
We are interested in studying slag deposits as unique habitat for microbial life. Slag samples originating from alum leaching were collected close to Bonn (Germany). Structure and genetic potential of the microbial communities residing in slag deposits were thoroughly characterized, in addition to slag geochemistry.
Slag deposits from alum leaching are highly eniched in aluminium, iron and sulfur. Low pH (~3.5) is a consequence of alum oxidation, thereby indicating a high bioavailability of aluminium, which is in its free form highly toxic. Alpha- and beta-diversity analyses indicated a strong selection for highly adapted microbial populations and multiple regression analyses identified aluminium to be the main driver for niche speciation. On phylum level, slag microbial communities were primarily composed of Acidobacteria (12-27%), Actinobacteria (20-35%), Chloroflexi (20-27%) and Alphaproteobacteria (13-18%). A more detailed analysis showed yet uncharacterized taxonomic groups to be most prominent: DA052 (up to 16%), KF-JG30-18 (up to 3%) [both Acidobacteria], TM214 (5-20%, Actinobacteria), DA111 (7-8%, Alphaproteobacteria) and JG37-AG-4 (15-20%, Chloroflexi). The assembly of taxonomically binned metagenome contigs allowed us to reconstruct more than 30 putative genomes, of which 10 were of sufficient quality for downstream analysis. These included Acidobacteria (5), Chloroflexi (3), Planctomycetes (1), Alphaproteobacteria (1), and Gammaproteobacteria (1). Apparently, slag microbes cope with high aluminium content by a combination of strategies, such as efflux mechanisms, chelation using organic acids and phospholipids, and aluminum-binding proteins known so far only from thermophilic organisms. In addition, high numbers of genes linked to heavy metal detoxification and xenobiotics breakdown are a common characteristic of these uncultured groups.
Slag deposits originating from mineral leaching represent unique habitats that allow studying aluminium tolerance and resistance at extreme geochemical settings. Learning more about how microbial communities adapt to these environmental conditions is of major interest given the ongoing acidification of arable soils worldwide.

Effects of different nanoparticles on soil microbial community structures and plant-microbe interactions

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Production of nanoparticles (NPs) is increasing for many different applications in which they are also released into the environment. In addition, they are also considered for agricultural use in plant protection products and fertilizers, which would result in even higher environmental loads, particularly in soils. Therefore, it is important to assess, whether NPs affect soil biology, such as microorganisms living in soil and crops growing in soil. In two pot experiments growing either wheat or red clover, we spiked agricultural soil with relatively high concentrations of different NPs, i.e., TiO_2 NPs, CeO_2 NPs and carbon nanotubes (CNTs). After three months, microbial community structures were assessed using direct DNA extraction from soil and Illumina MiSeq sequencing of PCR amplified ribosomal markers. Plant biomass, root colonization with arbuscular mycorrhizal fungi (AMF), as well as symbiotically fixed nitrogen (^{15}N) were also determined. In the wheat experiment, application of TiO_2 NPs altered the prokaryotic but not eukaryotic community structure. In the red clover experiment, prokaryotic community structure was not affected by TiO_2 and CeO_2 NPs, but altered by application of CNTs. As in the wheat experiment, eukaryotic microorganisms were not affected by any of the treatments. Wheat biomass increased by 6% in the TiO_2 treatment, while red clover biomass, N-fixation and AMF colonization were not affected. Only the CNT treatment showed increased N-fixation and 100% of the plant nitrogen was derived from symbiotic N-fixation. The results indicate that microbial communities can be affected by certain NPs, calling for further environmental risk assessments. In our experiments, prokaryotes were more sensitive to NPs than eukaryotes, which may relate to their different lifestyles but requires more detailed analyses. Further research is needed to test, whether effect sizes change if exposure time would be extended.

Responses of Thaumarchaeotal community in agricultural soils to acidification and polycyclic aromatic hydrocarbons contamination

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Thaumarchaeota are widespread in terrestrial environments and may contribute to soil ammonia oxidation. Accumulating evidence indicates that pH and pollutants such as toxic metals may substantially change the abundance, composition and activity of soil thaumarchaeota; however, the responses of thaumarchaeotal community to the co-occurrence of soil acidification and organic pollution have not been fully explored. In this study, field soils were collected from agricultural land impacted by fertilization-induced pH decline and polycyclic aromatic hydrocarbons (PAHs) contamination. Thaumarchaeotal abundance and community composition were assessed using molecular approaches targeting the 16S rRNA or *amoA* genes. The results demonstrate a significant correlation between thaumarchaeotal gene copies and soil pH, with the I.1a-associated group enriched in the samples of pH<5.0. Direct ordination analysis suggests that the variation in the thaumarchaeotal community was primarily related to soil pH. In contrast, only a significant correlation between PAHs and the abundance of thaumarchaeotal ammonia oxidizers was observed in these field soils. To evaluate the short-term responses of thaumarchaeota to PAHs stress, soil microcosms amended with selected PAHs were established. Despite the persistence of high concentrations of PAHs during the 4-week incubation, *amoA* abundance increased in comparison to the control microcosms, implicating the complex interactions between ammonia-oxidizing thaumarchaeota and organic pollutants in the soil. This study provides compelling evidence that soil pH variation strongly shapes the agricultural soil thaumarchaeotal community and, to our knowledge, represents the first attempt to assess the effects of PAHs on this ecologically relevant archaeal phylum.

Comparative phylogenetic analysis of bacterial community dynamics during multi-year bioremediation of oil-contaminated soil in a boreal climate

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The widespread use of motor oil makes it a notable risk factor to cause scattered contamination in soil. The monitoring of microbial community dynamics can serve as a comprehensive tool to assess the ecological impact of contaminants and their disappearance in the ecosystem. Hence, a field study was conducted to monitor the ecological impact of used motor oil under different perennial cropping systems (fodder galega, brome grass, galega-brome grass mixture and bare fallow) in a boreal climate zone. Bacterial overall communities and the prevalent sub-communities (e.g. classes Actinobacteria, Gammaproteobacteria and Betaproteobacteria), as determined by high-throughput sequencing, undergo changes following oil contamination over a four-year bioremediation period. The main contribution of this study is an increase in the knowledge about the salient bacteria taxa that discriminated the whole community between oil-contaminated and control soils at different phases of bioremediation using a comparative phylogenetic analysis. These taxa with positive response to oil contamination were assigned mostly to the two prevalent phyla Actinobacteria and Proteobacteria, revealing the importance of these bacterial groups in hydrocarbon degradation. Beside oil concentration, other soil parameters such as soil C, pH and EC and crop physiochemical parameters such as crop C and BNF yield explained small but significant proportions of bacterial community composition. Distinct differences in the composition and diversity of bacterial communities were found between vegetated plots and bare fallow in the third year 2012 after the perennial crops have established their stable growth, suggesting the importance of agricultural cultivation and soil management to alter the diversity and structure of soil-borne bacterial communities.

Molecular adaptation of the ammonia monooxygenase *amoA* gene during the ancient and rapid diversification of terrestrial Thaumarchaeota

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Thaumarchaeota form an ammonia-oxidizing archaeal phylum that is abundant in many ecosystems, including the soil, performing a key function in the global nitrogen cycle. Previous high throughput sequencing analysis of the ammonia monooxygenase gene *amoA* has demonstrated that pH is the major driver of thaumarchaeotal niche specialization and community structure of these organisms.

While many studies have examined the adaptive distribution and ecophysiology of extant Thaumarchaeota, the evolutionary rise of these prokaryotes to a position of ecological dominance in terrestrial habitats has been poorly considered. Therefore, we characterised thaumarchaeotal diversification with respect to ancestral reconstructions of soil pH adaptation, employing state-of-the-art comparative phylogenetic methods based on extensive *amoA* sequence data. Our analysis showed a striking increase in lineage diversification rates during early thaumarchaeotal evolution that was coupled to major pH adaptation events, while the high initial rate of diversification of Thaumarchaeota remained globally stable during the last 400-700 Ma.

To better understand the evolutionary processes involved in pH specialization during thaumarchaeotal diversification, this study aims to provide the first detailed analysis of molecular adaptation in the protein product *amoA* in the context of pH specialisation. A suite of probabilistic codon based approaches were applied to define instances of episodic and persistent positive selection and instances where selection has been relaxed. A combination of Bayesian phylogenetic approaches and ancestral protein and phenotypic reconstructions shows that repeated adaptation of AmoA is evident during specialisation of Thaumarchaeota in acidic soils but not in alkaline soils.

Altogether, this evolutionary study is the first of its kind to link phenotypic diversification with molecular adaptation in an abundant terrestrial prokaryotic phylum. This study provides a framework for understanding evolutionary processes and past molecular innovations in microbes.

Establishing dung fungal spores as a proxy for herbivore abundance: an experimental approach

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The past decades have seen an increase in the use of spores of coprophilous (dung-inhabiting) fungi from soil samples and sedimentary sequences as a proxy for past large herbivore presence and abundance. Although this novel proxy has great potential for advancing our understanding of past herbivore population dynamics, extinction events and the impact of husbandry practices on the natural environment, current research in this area is mostly qualitative and lacks a strong experimental basis. In particular, environmental and taphonomic factors influencing preservation and recovery of these spores are poorly understood.

I placed pollen (Tauber) traps at 11 locations in Chillingham Wild Cattle Park, Northumberland, UK, to investigate influx rates of coprophilous fungal spores. Soil and moss samples were also taken from the same locations to assess longer-term preservation and abundance of spores. A core from a small bog will provide a record for potentially the past millennia.

The park is inhabited by a herd of ~100 wild cattle, which have lived in the area since at least 1646 and receive no veterinary intervention. The park is also frequented by fallow and roe deer, as well as badgers and a variety of smaller mammals. The sampling locations represent a range of moisture levels (wet vs. dry), vegetation types (deciduous woodland, coniferous woodland and grassland) and animal densities. Two sampling periods for the pollen traps, one from October to March and one from April to September, will enable an assessment of seasonal variation in influx of coprophilous fungal spores.

I present preliminary results from a comparison between sampling sites with varying moisture levels, vegetation types and large herbivore densities to investigate the impact of environmental factors and animal densities on dung fungal spore representation in soil samples.

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Indirect and direct effects of long-term disturbance on above- and below-ground grassland communities

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Management strategies such as long-term inorganic fertilization and mowing change soil factors with direct and indirect effects on the plant and soil microbial communities. Here, we explored the effects of long-term nutrient addition with nitrogen (N), phosphorous (P) and NP-potassium (NPK), liming (L) or no fertilization (C) and twice yearly mowing on the plant, bacterial and fungal communities and soil physicochemical factors. We combined topsoil 16S rRNA and 18S rRNA gene surveys with plant frequency and edaphic factor measurements at the treatment level using multivariate statistics and co-inertia analysis. Treatment grouping had a significant effect on the communities and soil factors; the treatments affected the grassland components differently. Based on Between-Class Analysis (BCA), we found that the plant and fungal communities were distinct in the NPK and L treatments, while the bacterial communities and soil factors were distinct instead in the N and L treatments. Furthermore, we found significant co-structures between the plant and fungal but not between the plant and bacterial nor bacterial and fungal community compositions. Our findings support the idea that the soil bacterial community composition is influenced more by edaphic factors than by plant composition. Our results also suggest that plant and fungal communities are more tightly linked than either community with the bacterial community. We found potential links between the plant, bacterial and fungal taxa attributed to the fertilization treatments that can be explored in future works.

Improved P Acquisition in Crops by Inoculation with P-solubilising Microorganisms

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The harmful effects of chemical fertilizer inputs on environment and their high costs in agriculture crop production cannot be overlooked. Solubilisation of bounded Phosphorus (P) in low or high pH soils by microorganisms has been established as alternative to reduce chemical fertilizers input. Supported acidification from NH4 for enhance P solubilization by these microorganisms in buffered soil received less attention in monocot plants. In a Complete Randomized Blocked Design (RCBD), greenhouse experiments were conducted with maize using artificial substrate (70% sand and 30% soil from Karlsruhe, (Loese sub soil, pH 7.6, 25% CaCO3 CAL-P: 5 mg/kg soil)) and natural soil (30% sand and 70% organic farm low P soil from Klein Hohenheim, (clay-loam, pH 6.8, 20 mg CAL-P/kg soil)) on different N forms with microbial BIOEFFECTORS (BE's) for P solubilisation effects. In low buffer artificial, bio-effector Proradix enhanced the growth of maize plants supplied with rock phosphate as P and calcium nitrate as N while With N as ammonium sulfate, plant growth was also enhanced by Proradix. Increased substrate buffer by 25% CaCO3, N as ammonium sulfate showed more plant growth promotion (17% dry weight) than N as calcium nitrate. In the natural soil, the integrated approach using ammonium plus BE's had promising effects on shoot biomass and some mineral elements in shoot biomass of maize. This research is expected to reduce chemical fertilizer inputs by farmers increase yield and save the ecosystem.
Key words: rock phosphate (RP), bio-effectors, Phosphorus solubilizing microorganisms (PSM), integrated approach.

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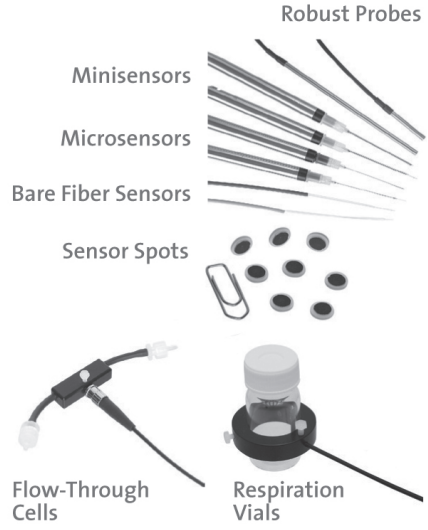

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