Four years of experimental climate change modifies the community structure of denitrifiers and the related microbial drivers of N2O fluxes in an upland grassland ecosystem.

Thomas Pommier, Amélie Cantarel, Juliette Bloor, Franck Poly

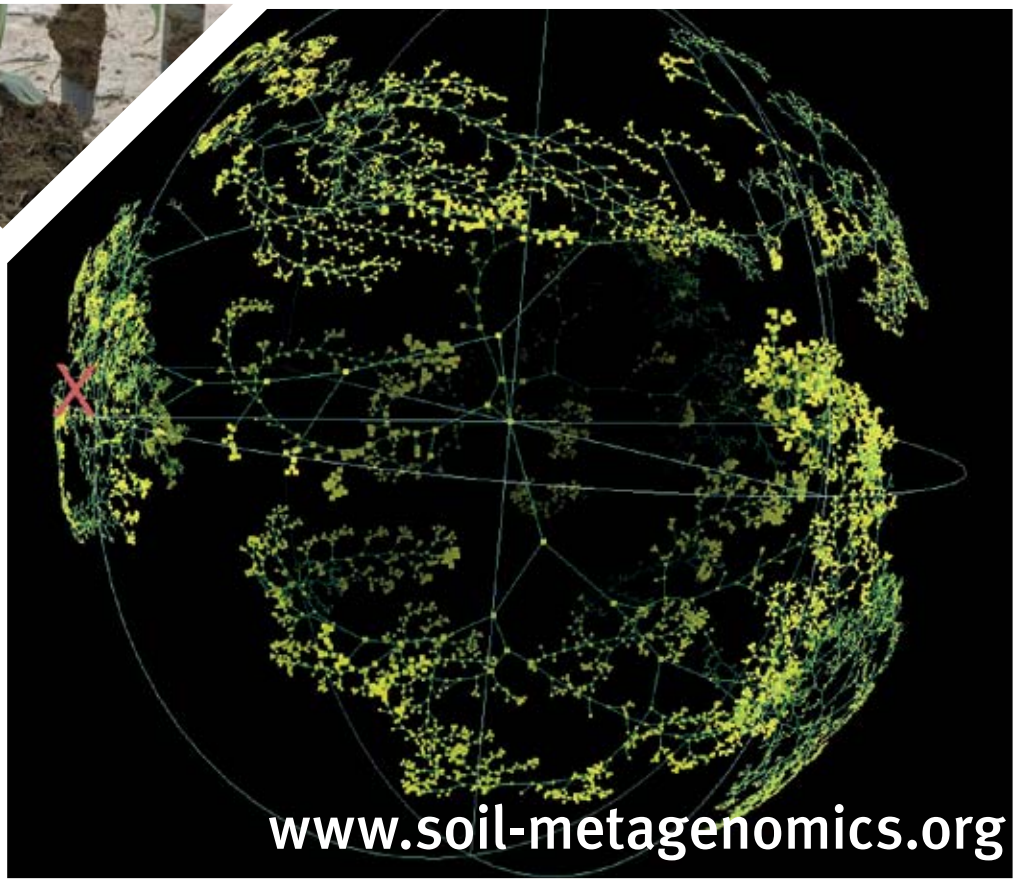
2nd THÜNEN SYMPOSIUM ON SOIL METAGENOMICS

MINING AND LEARNING FROM METAGENOMES

plus Workshop on Bioinformatic Tools

December 11–13, 2013

Braunschweig, Germany



THÜNEN

PROGRAMME

Scientific Programme Overview

THÜNEN

	Wednesday, 11 December 2013	Thursday, 12 December 2013	Friday, 13 December 2013
08:00			
	08:30–10:00 Workshop on bioinformatic tools: Part I – working with amplicon sequences I		
09:00		09:00–10:45 Drivers of soil biodiversity	09:00–10:45 The flexible metagenome
10:00	Industrial exhibition & coffee break		
	10:25–11:40 Workshop on bioinformatic tools: Part I – working with amplicon sequences II	Industrial exhibition & coffee break	Industrial exhibition & coffee break
11:00		11:10–12:55 Impacts of agriculture and climate change	11:10–12:40 From ecology to biotechnology
12:00	Lunch break *only for workshop participants*		12:40–13:30 Emerging issues & final discussion
13:00	13:00–13:15 Opening of Symposium	Industrial exhibition & lunch break	Industrial exhibition & lunch break
	13:15–15:00 News from the bioinformatic toolbox		
14:00		14:00–15:30 Linking structure to function I	14:30–15:30 Workshop on bioinformatic tools Part II – assembly of Metagenomes I
15:00	Industrial exhibition & coffee break		
	15:25–17:10 Unraveling biodiversity	Industrial exhibition & coffee break	Coffee break
16:00		15:55–17:25 Linking structure to function II	15:45–17:30 Workshop on bioinformatic tools Part II – assembly of Metagenomes II
17:00	17:10–19:00 Posters & drinks		
		17:25–19:15 Posters	
18:00			
19:00		19:15–19:30 Bright surprise @ Thünen Forum	
		19:30-21:30 Conference buffet @ Thünen Forum	
20:00			
21:00			

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Venue and Date
Thünen Institute, Forum
Bundesallee 50
38116 Braunschweig (Germany)
December 11–13, 2013

Conference Website
www.soil-metagenomics.org

Conference Chair
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Bundesallee 50
38116 Braunschweig (Germany)

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Welcome Note of the Conference Chair

Dear colleagues,

Johann Heinrich von Thünen, the name patron of the Thünen Institute, already recognized in the early 19th century the economic value of the "richness of soil", a characteristic he defined by its agricultural productivity. In a world which needs to feed 10 billion people in the near future, while at the same time loosing large areas of fertile land and facing increasingly limited resources of fertilizers, soil richness for agricultural productivity becomes a very modern, if not the key issue for our future survival. A huge scientific and political effort is required to develop an agriculture which produces more and which is at the same time protective to its natural resources, including the richness of our soils.

As microbiologists we know that a huge potential to address these challenges is hidden in the soil and its indigenous microbial inhabitants. Soil microorganisms sustain nutrient cycling, promote plant growth, inhibit plant diseases, and have the potential to produce bio-energy and products of outstanding values, including industrial enzymes, bio-pesticides and antibiotics. Much of our understanding of soil microorganisms, however, is still based on the relatively few members of the soil microbial community, which happen to be easily culturable on laboratory growth media.

Facing these new challenges of increasing agricultural productivity in a sustainable way, a much deeper exploration and understanding of the total soil microbial communities and interactions as well as their responses to environmental changes is now required. Soil metagenomics and its sisters (metatranscriptomics, -proteomics, -metabolomics), which together directly look into the bio-molecular composition of soils, come at the right moment. First examples already demonstrate the importance of these new scientific fields for biotechnology, ecology and agriculture.

The objective of this 2nd Thünen Symposium on Soil Metagenomics is to connect bioinformatics to microbial ecology research. The speed and cost efficiency of today's DNA and RNA sequencing technologies now requires an effort for a corresponding response from soil microbial ecology. Vice versa, the requirements to characterize microbial communities and address ecologically relevant questions will instruct further bioinformatic efforts, e.g., for sound statistical evaluations and visualizations of results. This Thünen Symposium is meant as an instrument for communication and learning from each other. For the first time, the Thünen Symposium is flanked by a workshop on bioinformatic tools in which applications for nucleic aid analyses are demonstrated, shared and discussed.

Welcome in Braunschweig, and thank you for your contribution to make this an exciting event.

Prof. Dr. Christoph C. Tebbe

08^{30}–11^{40}	**Workshop on bioinformatic tools – Part I** **Working with amplicon sequences**
Chair	Folker Meyer (Argonne, IL/US)
08^{30}	Welcome Anja Dohrmann (Braunschweig/DE)
08^{35}	Introduction Folker Meyer (Argonne, IL/US)
08^{45} WS 01	The basics of amplicon processing Martin Hartmann (Zurich/CH)
10^{00}–10^{25}	Industrial exhibition and coffee break
10^{25} WS 02	SILVA and SILVA NGS – practical guidance Frank Oliver Glöckner (Bremen/DE)
11^{10} WS 03	What's the Cloud but for water? Folker Meyer (Argonne, IL/US)
11^{40}–12^{30}	Lunch break (only for Workshop participants)

13^{00}–13^{15}	**Opening of the Symposium** Christoph C. Tebbe, Organizer, Thünen Institute of Biodiversity Folkhard Isermeyer, President of the Thünen Institute
13^{15}–15^{00}	**News from the bioinformatic toolbox**
Chair	Pascal Simonet (Lyon/FR)
13^{15} KN1	Environmental Bioinformatics – with a focus on "Marine" Frank Oliver Glöckner (Bremen/DE)
13^{45} KN2	Current bioinformatic challenges in soil shotgun metagenomics Folker Meyer (Argonne, IL/US)
14^{15} OR1	NoDe and CATch – two algorithms to get more accurate 16S rRNA sequencing data Mohamed Mysara (Brussels/BE)
14^{30} OR2	Expanding the bioinformatic toolbox for the analysis of complex metagenomes Kostas Konstantinidis (Atlanta, GA/US)
14^{45} OR3	Assembly of a soil derived microbial consortium using a massive shared-memory computational system reveals novel bio-industrial enzymes Alexander John Ropelewski (Pittsburgh, PA/US)
15^{00}–15^{25}	Industrial exhibition and coffee break
15^{25}–17^{10}	**Unraveling biodiversity**
Chair	James Prosser (Aberdeen/GB)
15^{25} KN3	Fungal communities in soils – structure, dynamics and functioning Petr Baldrian (Prague/CH)
15^{55} KN4	Metagenomics of the rhizosphere Jim Tiedje (East Lansing, MI/US)
16^{25} OR4	Taxonomical and functional microbial community selection in soybean rhizosphere Eiko Kuramae (Wageningen/NL)
16^{40} KN5	Reconstruction of microbial nutrient cycles in soil using metagenomic approaches Michael Schloter (Munich/DE)
17^{10}–19^{00}	**Posters and drinks**

09^{00}–10^{45}	**Drivers of soil biodiversity**
Chair	Jim Tiedje (East Lansing, MI/US)
09^{00} KN6	A cross-site investigation of belowground community responses to shifts in nutrient availability Noah Fierer (Boulder, CO/US)
09^{30} KN7	Turnover of soil microbial diversity is driven by wide-scale environmental heterogeneity Nicolas Chemidlin (Dijon/FR)
10^{00} OR5	Spatial structure of soil microbial communities from centimeters to ecosystems Sarah O´Brien (Argonne, IL/US)
10^{15} OR6	Microscale patterns of bacterial diversity George Kowalchuk (Utrecht/NL)
10^{30} OR7	Spatial analysis of bacterial communities in soil – the effect of distance and environmental perturbation Jean-Sébastien Beaulne (Ecully/FR)
10^{45}–11^{10}	Industrial exhibition and coffee break
11^{10}–12^{55}	**Impacts of agriculture and climate change**
Chair	Michael Schloter (Munich/DE)
11^{10} KN8	Soil microbial community responses to warming as revealed by Metagenomics Kostas Konstantinidis (Atlanta, GA/US)
11^{40} OR8	Revealing microbial climate control – ecophysiology of sulphate-reducing microorganisms in peatlands Bela Hausmann (Vienna/AT)
11^{55} OR9	Microbial diversity of dryland soils and its role in greenhouse gas turnover Roey Angel (Vienna/AT)
12^{10} OR10	Integrating “omics” to understand soil C cycling responses to precipitation variation David Myrold (Corvallis, OR/US)
12^{25} OR11	Long-term effects of N, P, and K fertilization on the composition and function of bacterial communities in grassland soil Noriko Cassman (Wageningen/NL)
12^{40} OR12	The response of soil microbial diversity to long-term organic and conventional farming Martin Hartmann (Zurich/CH)
12^{55}–14^{00}	Industrial exhibition and lunch break

14^{00}–15^{30}	**Linking structure to function l**
Chair	Jan Dirk van Elsas (Groningen/NL)
14^{00} KN9	Multi-substrate isotope labeling and metagenomic analysis of active soil bacterial communities Josh D. Neufeld (Waterloo/CA)
14^{30} OR13	Organic carbon mineralisation in arctic peatsoils – key functions and microorganisms Tim Urich (Vienna/AT)
14^{45} OR14	Comparative metagenomics of disease suppressive soils Allison Jack (Wageningen/NL)
15^{00} OR15	Habitat filtering and competitive exclusion of large clades underlie phylogenetic clustering in soil bacteria Marta Goberna (Moncada/ES)
15^{15} OR16	The strength of phylogenetic signals in microbial traits – a proof of principle as applied to methane-oxidizing bacteria Sascha Krause (Wageningen/NL)
15^{30}	Industrial exhibition & coffee break
15^{55}–17^{25}	**Linking structure to function ll**
Chair	David Myrold (Corvallis, OR/US)
15^{55} OR17	Reconstruction of full-length SSU rRNA genes from DNA/RNA-sequ data profiles the three domains of life in soil environments Ulisses Nunes da Rocha (Berkeley, CA/US)
16^{10} OR18	Functional soil metagenomics – elucidation of polycyclic aromatic hydrocarbon degradation potential after 10 years of in situ bioremediation Marcia Duarte (Braunschweig/DE)
16^{25} OR19	Cultivation and metaomics approaches characterize organohalide-respiring communities Frank Loeffler (Oak Ridge, TN/US)
16^{40} OR20	The evolutionary space of the bacterial 16S rRNA gene as a new operational field for integral metagenomics Evgeny Andronov (St. Petersburg/RU)
16^{55} KN10	Metamoanics, lost watches and glimmers of hope James Prosser (Aberdeen/GB)
17^{25}–19^{15}	Posters
19^{15}	Bright surprise @ Thünen Forum
19^{30}	Conference buffet @ Thünen Forum (see p. 25)

09^{00}–10^{45}	**The flexible metagenome**
Chair	Gabriele Berg (Graz/AT)
09^{00} KN11	Trends and barriers for plasmid- and phage- mediated lateral gene transfer Tal Dagan (Kiel/DE)
09^{30} KN12	Horizontal transfer of short DNA sequences Kaare M. Nielsen (Tromsø/NO)
10^{00} OR21	Amazonian resistome – evaluating antibiotic resistance abundance through metagenomic approaches Joseph Nesme (Ecully/FR)
10^{15} OR22	Unraveling the diversity and dynamics of the bacterial mobilome in on-farm biopurification systems Kornelia Smalla (Braunschweig/DE)
10^{30} OR23	Production of secondary metabolites in soil and sand microcosms of *Streptomyces coelicolor* Geertje van Keulen (Swansea/GB)
10^{45}	Industrial exhibition & coffee break
11^{10}–12^{40}	**From ecology to biotechnology**
Chair	Kornelia Smalla (Braunschweig/DE)
11^{10} KN13	Combination of conceptual and technical approaches for exploiting soil microbial resources Pascal Simonet (Lyon/FR)
11^{40} KN14	Mining large-insert soil metagenomic libraries for antimicrobial activity and biosynthetic pathways Mark R. Liles (Auburn, AL/US)
12^{10} OR24	Genomics and metagenomics for discovery of novel bacterial laccases Ines Mandic-Mulec (Ljubljana/SI)
12^{25} OR25	Next-generation biocontrol products – development and optimization by meta-omics technologies Gabriele Berg (Graz/AT)
12^{40}–13^{25}	**Emerging issues for soil Metagenomics – open discussion**
Chair	Timothy M. Vogel (Lyon/FR)
12^{40} OR26	Emerging issues – an opinion Timothy M. Vogel (Lyon/FR)
12^{55}	Open stage and discussion
13^{25}	**Farewell and announcements** Christoph Tebbe
13^{30}–14^{30}	Industrial exhibition and lunch break

14^{30}–17^{30}	**Workshop on bioinformatic tools – Part II** **Assembly of Metagenomes**
Chair	Martin Hartmann (Zurich/CH)
14^{30} WS4	PANDAseq PE assembly, QIIME/AXIOME with indicator species and MRPP, SSUnique Josh D. Neufeld (Waterloo/CA)
15^{30}–15^{45}	Coffee break
15^{45} WS5	Understanding and using MG-RAST API, Kbase, and Metagenomic Assembly Folker Meyer (Argonne, IL/US)
17^{30}	**End of Symposium**

The poster sessions are scheduled on Wednesday, 11 December 2013 from 17^{10}-19^{00} hrs. and on Thursday, 12 December 2013 from 17^{25}-19^{15} hrs.

The following poster presentations will be held during these sessions:

Poster Session I • Wednesday, 11 December 2013 (17^{10}–19^{00} hrs)

P1-P11 (except P2) Bioinformatic tools (see page 13)

P12-P21 Capturing nucleic acids from soil (see pages 13 ff.)

P22-P45 Impact of agriculture and climate change (see pages 14 ff.)

P46-P55 Mobile DNA and antibiotic resistances (see pages 15 ff.)

P56-P63 Organic waste treatments, aerosols and bioenergy (see pages 16 ff.)

P64-P70 Spatial ecology and biogeography (see pages 16 ff.)

P71-P78 Stability and vulnerability of microbial communities (see pages 17 ff.)

Poster Session II • Thursday, 12 December 2013 (17^{25}–19^{15} hrs)

P2 Bioinformatic tools (see page 19)

P79-P149 Structural and functional microbial diversity (see pages 19 ff.)

P150-P151 Biocontrol (see page 23)

Please note that all posters from session I should be hanging on Wednesday by 17^{00} hrs. and be removed at the latest by Thursday 11^{00} hrs.
Posters of session II should be hanging on Thursday by 17^{00} hrs. and be removed at the latest by Friday 11^{00} hrs.
Posters that have not been removed by that time will be considered as waste.

The poster sessions will take place in the basement of the Forum. Please follow the signs!

Bioinformatic tools

P1 CopyRighter, improved quantification in microbial amplicon surveys through gene copy number correction
Florent Angly, Paul Dennis, Adam Skarshewski, Inka Vanwonterghem, Phil Hugenholtz, Gene Tyson (Brisbane/AU)

P3 GnS-PIPE – an optimized bionformatic pipeline to efficiently assess microbial taxonomic diversity of complex environments using high throughput sequencing technologies
Sebastien Terrat, Samuel Dequiedt, Mélanie Lelièvre (Dijon/FR), Richard Christen (Nice/FR)
Pierre-Alain Maron, Lionel Ranjard (DIJON/FR)

P4 A workflow for analyzing taxon and pathway coverage profiles with applications to metatranscriptomics
Daniela Beisser, Inken Wohlers, Johannes Köster, Sven Rahmann (Essen/DE)

P5 Design and experimental validation of a novel non-degenerate universal primer set for 16S rRNA gene amplicon sequencing with a low possibility to amplify eukaryotic rRNA genes
Hiroshi Mori (Yokohama, Ookayama/JP), Fumito Maruyama (Tokyo/JP), Hiromi Kato (Sendai/JP)
Atsushi Toyoda (Shizuoka/JP), Ayumi Dozono (Yokohama/JP), Yoshiyuki Ohtsubo, Yuji Nagata (Sendai/JP)
Asao Fujiyama (Shizuoka, Tokyo/JP), Masataka Tsuda (Sendai/JP), Ken Kurokawa (Yokohama, Ookayama/JP)

P6 RDP – data and tools for soil microbial ecology
James Cole, Qiong Wang, Jordan Fish, Benli Chai, C. Titus Brown, Yanni Sun, James Tiedje (East Lansing, MI/US)

P7 Habitat specific adaptation of alphaproteobacteria based on GC content, genome size and aromatic dioxygenase gene distribution
Sajan Raju, Kim Yrjälä (Helsinki/FI)

P8 Mixture models for the estimation of metagenomic abundances
Kathrin Aßhauer, Heiner Klingenberg, Thomas Lingner, Peter Meinicke (Göttingen/DE)

P10 A pipeline for the analysis of MDA-based metagenomics read sets
Giorgio Gonnella, Mirjam Perner, Stefan Kurtz (Hamburg/DE)

P11 Extracting Genomes from Metagenomes, a study on permafrost soils in Barrow, Alaska
Aviaja Hauptmann, Jacob Bælum, Nikolaj Blom (Lyngby/DK), Neslihan Tas, Janet Jansson (Berkeley, CA/US)

Capturing nucleic acids from soil

P12 Metagenomic approach applied to the microbial community of a saline soil with high spatial variability
Loredana Canfora (Rome/IT), Giovanni Bacci (Firenze/IT), Flavia Pinzari (Rome/IT), Giuseppe Lo Papa (Rome, Palermo/IT)
Anna Benedetti (Rome/IT)

P13 Vascular wilt in lamb´s lettuce – metagenomic analysis of soil microbiota to identify relevant organisms
Katharina Piel, Annette Reineke (Geisenheim/DE)

P14 Functional screening of a metagenomic library for aerobic toluene degradation genes
Emna Bouhajja (Louvain la neuve/BE), Isabelle F. George (Brussels/BE), Rob Van Houdt (Mol/BE)
Mark R. Liles (Alabama, AL/US), Spiros N. Agathos (Louvain la neuve/BE)

P16 Simultaneous DNA, RNA, and protein extraction from a well-characterized soil for genomic and proteomic applications
Heather Callahan, Suzanne Kennedy (Carlsbad, CA/US), Iratxe Zarraonaindia (Argonne/FR; Bilbao/ES)
Jarrad Hampton-Marcell, Sarah Owens (Argonne/FR; Chicago, IL/US), Khatereh Motamedchaboki (La Jolla, CA/US)
Jack Gilbert (Argonne/FR; Chicago, IL/US)

P17 Microbial community potentially responsible for acid and metal release from an Ostrobothnian acid sulfate soil
Xiaofen Wu, Zhen Lim Wong (Kalmar/SE), Pekka Sten, Sten Engblom (Vaasa/FI), Peter Österholm (Åbo/FI)
Mark Dopson (Kalmar/SE)

P18 Beyond the soil dirt – optimizing a RNA-based approach to study microbial communities
Inês Nunes, Samuel Jacquiod, Anders Priemé, Søren Sørensen (Copenhagen/DK)

P20 The compound G2 increases yield of DNA extraction from clayey sediments up to 40X
Tue Kjærgaard Nielsen (Copenhagen/DK), Jacob Bælum (Lyngby/DK), Carsten Suhr Jacobsen (Copenhagen/DK)

P21 Using functional metagenomics for the discovery of new antimicrobial compounds
Gregory Amos, Chiara Borsetto, Nikos Kyratsous, Paris Laskaris, David Hodgson (Coventry/GB)
David Pearce (Cambridge/GB), Christophe Corre, Elizabeth Wellington (Coventry/GB)

Impact of Agriculture and Climate Change

P22 Fungal specific response in decomposing sugarcane leaf litter to no-tillage and bagasse mulching practices determined by Ion torrent ITS amplicon sequencing
Toshiko Miura (Yokohama/JP), Ainin Niswati, I. Gede Swibawa, Sri Haryani, Heru Gunito (Lampung/ID)
Nobuhiro Kaneko, Koichi Fujie (Yokohama/JP), Satoshi Shimano (Sendai/JP)

P23 Four years of experimental climate change modifies the community structure of denitrifiers and the related microbial drivers of N_2O fluxes in an upland grassland ecosystem
Thomas Pommier, Amélie M. Cantarel (Villeurbanne/FR), Juliette M. Bloor (Clermont-Ferrand/FR), Franck Poly (Villeurbanne/FR)

P24 Influence of tillage and fertilizer type on N cycling microbial populations
Hans-Martin Krause, Maike Krauss, Simone Spangler, Cecile Thonar (Frick/CH), Reiner Ruser (Frick/CH; Hohenheim/DE)
Andreas Gattinger, Paul Mäder (Frick/CH)

P25 Agricultural impacts upon the soil microbiome:the implications on carbon cycling.
Paul Flanagan (Belfast/IE), Kris Hart (London/GB), Brian Murphy (Dublin/IE), Alessandra Frau, Christopher Allen (Belfast/IE)
Andre Simpson (Toronto/CA), Brian Kelleher (Dublin/IE)

P26 Effects of N deposition on diazotrophic activity and distribution of microorganisms associated with *Sphagnum magellanicum*
Martine Kox, Katharina Ettwig, Leon Lamers, Eva van den Elzen, Christian Fritz (Nijmegen/NL)

P27 Dominant bacteria are responsive to 20+ years of experimental soil warming in a mixed deciduous forest
Kristen M. DeAngelis, Grace Pold (Amherst, MA/US)

P28 Monocropping drives the homogenization of bacterial communities at a regional scale in no-till fields of Argentinean pampas
Eva Figuerola, Leonardo Erijman, Leandro Guerrero, Dominique Türkovsky, Luis Wall (Buenos Aires/AR)

P29 Non-target effects of chemical v/s bio-pesticides on rhizospheric microbial diversity
Shilpi Sharma, Sunil Singh, Sukriti Gupta, Rashi Gupta (New Delhi/IN)

P30 Impact of agricultural practices on soil microbial communities in a loamy soil in Belgium
Florine Degrune, Aurore Stroobants (Gembloux/BE), Bernard Taminiau (Liège/BE), Carine Nezer (Herstal/BE)
Georges Daube (Liège/BE), Micheline Vandenbol (Gembloux/BE)

P31 Plant species composition and season influence shifts on the soil microbial community composition in response to climate change
Tesfaye Wubet, Beatrix Schnabel, Sigrid Haertling, Thomas Reitz, Elke Schulz, Mika Tarkka (Halle a. d. S./DE)
Alexandra Weigelt (Leipzig/DE), Francois Buscot (Halle a. d. S., Leipzig/DE)

P32 Effect of long term differential fertilization on the soil microbial eukaryotic community – a metagenomic analysis
Guillaume Lentendu (Halle a. d. S., Leipzig/DE), Tesfaye Wubet (Halle a. d. S./DE), Antonis Chatzinotas, Susann Müller
Christian Wilhelm (Leipzig/DE), François Buscot (Halle a. d. S., Leipzig/DE), Martin Schlegel (Leipzig/DE)

P33 Changes in bacterial community structure as a response to drought stress in forest ecosystems – a roof experiment
Katja Felsmann, Arthur Gessler, Andreas Ulrich (Müncheberg/DE)

P34 Infection dynamics of insecticide-degrading symbionts in the bean bug Riptortus pedestris – Which bacteria dominate in insecticide-sprayed soil and then infect to the bugs?
Hideomi Itoh (Hokkaido/JP), Tomo Aoyagi, Ronald Navarro, Yuya Sato, Kanako Tago, Masahito Hayatsu
Tomoyuki Hori (Tsukuba/JP), Yoshitomo Kikuchi (Hokkaido/JP)

P35 Endophytic bacterial diversity in Eucalyptus and the influence of mixed plantation and N addition
Raquel Peixoto, Eduardo Fonseca, Caio Rachid, Fabiano Balieiro, Alexandre Rosado, Guilherme Chaer
James Tiedje (Rio de Janeiro/BR)

P36 Plant coverage determine the fungal community structure and diversity in a forestry system
Alexandre Rosado, Caio Rachid, Fabiano Balieiro, Eduardo Fonseca, Raquel Peixoto, Guilherme Chaer
James Tiedje (Rio de Janeiro/BR)

P37 Assessing nitrous oxide emissions and microbial structure after sugarcane fertilization
Leonardo Pitombo (Wageningen/NL; Sorocaba/BR), Janaina Carmo (Sorocaba/BR)
Johannes van Veen (Wageningen/NL), Heitor Cantarella (Campinas/BR), Eiko Kuramae (Wageningen/NL)

P38 The Arctic snowpack microbial community highlighted by metagenomics and metatranscriptomics
Lorrie Maccario, Catherine Larose, Timothy Vogel (Ecully/FR)

P39 Unravelling keyplayer denitrifiers and dynamics of transcription activity in high and low pH soils
Binbin Liu, Natalie Lim, Lars Bakken, Åsa Frostegård (Aas/NO)

P40 Pesticides influence on bacterial communities from expired pesticides landfills
Marcin Golebiewski (Torun, Warsaw/PL), Marcin Ostajewski, Michal Kaminski, Pawel Krawczyk, Adam Sobczak
Leszek Lipinski (Warsaw/PL)

P41 Cattle outdoor husbandry results in the introduction and survival of rumen-borne *Archaea* and *Bacteria* into the intensively pastured soil
Alica Chronakova (Ceske Budejovice/CZ), Brigitte Hai (Munich/DE), Christopher Quince (Glasgow/GB)
Dana Elhottova, Miloslav Simek (Ceske Budejovice/CZ), Michael Schloter (Munich/DE)

P42 Predictive model of soil molecular microbial biomass by using the French soil quality monitoring network data
Walid Horrigue, Lionel Ranjard, Samuel Dequiedt (Dijon/FR)

P43 Amazonian deforestation alters bacterial networks in soil
Acacio Navarrete (Piracicaba/BR), Eiko Kuramae (Wageningen/NL), Siu Mui Tsai (Piracicaba/BR)
Lucas Mendes (Piracicaba/BR), Mattias de Hollander, Johannes van Veen (Wageningen/NL)

P44 Effect of saline irrigation water on the diversity of indigenous microbial communities in soils from a semi-arid ecosystem
Astrid Näther (Braunschweig/DE), Angel Carrillo, Thelma Castellanos (La Paz/MX), Anja B. Dohrmann, Kornelia Smalla
Christoph C. Tebbe (Braunschweig/DE)

P45 Importance of land use change for microbial diversity in European soils
Anja Bettina Dohrmann, Axel Don, Christopher Poeplau, Christoph C. Tebbe (Braunschweig/DE)

Mobile DNA and antibiotic resistances

P46 Exploring the prokaryotic insertion sequence abundance and diversity of brazilian sugar-cane cultivated soils
Alessandro Varani, Eliamar Aparecieda Nascimento Pedrinho, Camila Fernandes, Alessandra dos Santos Pinto
Eliana Gertrudes de Macedo Lemos (Jaboticabal/BR)

P47 Metagenomics unravels the antibiotic resistome of Indian soils
Johan Bengtsson-Palme, Erik Kristiansson, Joakim Larsson (Göteborg/SE)

P48 Gene transfer from genetically modified plants to micro-organisms
Sandrine Demaneche, Alban Mathieu, Pascal Simonet (Ecully/FR)

P49 Effect of amendments with excrements from CTC-treated cows on bacterial community structure in pasture soils
Alica Chronakova, Martina Kyselkova, Dana Elhottova (Ceske Budejovice/CZ)

P50 Broad host range vectors for expression of proteins with (Twin-) Strep-tag, HIS-tag and engineered, export optimized Yellow Fluorescent Protein
Thorben Dammeyer (Braunschweig/DE)

P51 Plasmid pool in pesticide-contaminated soils
Pawel Krawczyk, Ewa Lewczuk, Dorota Adamska, Leszek Lipinski, Adam Sobczak, Andrzej Dziembowski (Warsaw/PL)

P52 Quantification of plasmids in soils stressed by different conditions
Claudia I. De La Cruz-Perera (Copenhagen/DK; Ecully/FR), Søren J. Sørensen (Copenhagen/DK)
Tim M. Vogel (Copenhagen/DK; Ecully/FR), Sandrine Demaneche (Ecully/FR)

P53 Evaluating the mobilome of environmental samples through metagenomics
Nicole Ricker (Toronto/CA), Shu Yi (Roxana, IL/US) Shen, Roberta Fulthorpe (Toronto/CA)

P54 Amplicon sequencing and resistance gene pool of bacterial communities from wastewater irrigation fields in the Mezquital Valley, Mexico
Melanie Broszat (Freiburg/DE), Heiko Nacke (Göttingen/DE), Ronja Blasi (Freiburg/DE)
Christina Siebe (Mexico City/MX), Johannes Huebner (Freiburg/DE), Rolf Daniel (Göttingen/DE)
Elisabeth Grohmann (Freiburg/DE)

P55 Widespread dissemination of class 1 integron components in soils and related ecosystems as revealed by cultivation-independent analysis
Sven Jechalke, Susanne Schreiter, Birgit Wolters, Simone Dealtry, Holger Heuer, Kornelia Smalla (Braunschweig/DE)

Organic Waste Treatments, Aerosols and Bioenergy

P56 Biodegradation of polyester polyurethane buried under compost at different temperatures
Urooj Zafar, Geoff Robson (Manchester/GB)

P57 Selection and metataxonomic characterization of soil microbial communities involved on wheat straw bioconversion
Diego Javier Jimenez Avella, Francisco Dini Andreote, Jan Dirk van Elsas (Groningen/NL)

P58 The role of mobile genetic elements in pesticide biodegradation in on- farm biopurification systems
Basak Ozturk, Vincent Dunon, Karolien Bers, Rene de Mot (Leuven/BE), Kornelia Smalla (Braunschweig/DE)
J.S Sorensen (Copenhagen/DK), Rob Lavigne, Dirk Springael (Leuven/BE)

P59 Methane production from microalgae at high and at normal pH – metagenomic and metatranscriptomic analyses of alkaline and mesophilic biogas reactors
Halina Tegetmeyer, Vímac Nolla-Ardèvol, Regina Vahrenhorst (Bielefeld/DE), Marc Strous (Calgary/CA; Bremen/DE)

P60 Potential of petroleum hydrocarbon biodegradation from Trindade Island coastal soils, Brazil
Daniel Kumazawa Morais, Patricia Ciacco Gianelli, Victor Satler Pylro, Marcos Tótola (Viçosa/BR)

P61 Metagenomic/transcriptomic analysis of the cathode associated community of a bioelectrical system
Brian Eddie, Zheng Wang, W. Judson Hervey, IV, Anthony P. Malanoski, Sarah M. Strycharz-Glaven
Baochuan Lin (Washington D.C., WA/US)

P62 Group-specific pyrosequencing to identify low abundant Clostridia in a biogas plant
Anja Bettina Dohrmann (Braunschweig/DE), Meike Walz, Achim Loewen (Göttingen/DE)
Christoph C. Tebbe (Braunschweig/DE)

Spatial Ecology and Biogeography

P64 Edaphic, environmental and spatial drivers of microbial communities of Australia soils
Andrew Bisserr, Kelly Hamonts, Andrew Young (Canberra/AU)

P65 Soil Bacterial Biogeography in the Western Swiss Alps
Erika Yashiro, Eric Pinto, Antoine Guisan, Jan Roelof van der Meer (Lausanne/CH)

P67 Expanding the soil fungal diversity through barcoding and illumina paired-end of the ITS1 region
Eric Pinto, Aline Buri, Erika Yashiro, Jan van der Meer, Helene Hirzel, Antoine Guisan (Lausanne/CH)

P68 Soil hydrophobicity and greenhouse gas flux dynamics – an integrated approach
Khalid Qassem, Emilia Urbanek, Geertje van Keulen (Swansea/GB)

P69 Illumina metabarcoding of a soil fungal community
Philipp-André Schmidt, Miklós Bálint, Bastian Greshake (Frankfurt a. M./DE), Cornelia Bandow, Jörg Römbke (Flörsheim/DE)
Imke Schmitt (Frankfurt a. M./DE)

P70 Pre-evaluation of an old creosote polluted site for phytoremediation – spatial variation of PAH contamination, geochemical properties and microbial communities
Kim Yrjälä, Shinjini Mukherjee, Pauli Siivonen, Pirjo Tuomi (Helsinki/FI), Pertti Pulkkinen (Läyliäinen/FI)

Stability and Vulnerability of Microbial Communities

P71 Microbial ecology of exotic plant invasions
Sean Gibbons (Chicago, IL/US; Argonne/FR), Ylva Lekberg, Dan Mummey, Philip Ramsey (Missoula, MT/US)
Jack Gilbert (Argonne/FR; Chicago, IL/US)

P72 Microbiome impact assessment of iron oxide nanoparticles used for bioremediation of hydrocarbon contaminated aquifers
Béatrice Frank-Fahle, Giovanni Pilloni (Neuherberg/DE), Sebastian Höss (Starnberg/DE), Tillmann Lüders (Neuherberg/DE)

P73 Shifts in microbial communities in response to long-term silver exposure
Sotirios Vasileiadis, Edoardo Puglisi, Marco Trevisan (Piacenza/IT), Kate Langdon, Mike McLaughlin, Enzo Lombi
Erica Donner (Adelaide/AU)

P74 Microbial community structure at habitat level controls the response to antibiotics
Rüdiger Reichel, Diana Patzelt, Christoph Barleben (Trier/DE), Ingrid Rosendahl (Bonn/DE)
Ruth H. Ellerbrock (Müncheberg/DE), Sören Thiele-Bruhn (Trier/DE)

P75 A temporal metagenomic survey of soil microbial community following willow planting in petroleum hydrocarbon-contaminated soil
Yves Terrat, Sebastien Halary, Etienne Yergeau, Mohamed Hijri, Marc St-Arnaud (Montreal/CA)

P76 Comparative Metagenomics unravel adaptive evolution processes in *Dehalococcoides mccartyi*
Burcu Simsir (Knoxville, TN/US), Despina Tsementzi, Kostas T. Konstantinidis (Atlanta, GA/US)
Frank E. Loeffler (Knoxville, TN/US)

P77 The influence of Acacia invasion and clearing methods on bacterial communities in Fynbos soil
Etienne Slabbert, Karin Jacobs (Stellenbosch/ZA)

P78 Conceptual and methodological framework to manage microbial robustness using molecular systems synecology: applications for the biodegradation of contaminants of emerging concern
Benoit Stenuit, Spiros N. Agathos (Louvain-la-Neuve/BE)

Bioinformatic tools

P2 StreamingTrim 1.0 – a Java software for dynamic trimming of 16SrRNA sequence data from metagenetic studies
Giovanni Bacci, Marco Bazzicalupo (Florence/IT), Anna Benedetti (Rome/IT), Alessio Mengoni (Florence/IT)

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P79 Analysis of methane production and methanogenic community across rice cultivars
Suresh Dubey, Alpana Singh, Navnita Srivastva (Varanasi/IN)

P80 Solubilisation of iron ore mineral by a fungus
Rasheed Adeleke, Mphekgo Maila (Pretoria/ZA)

P81 Increasing management intensity reduces the diversity of arbuscular mycorrhizal fungi on Arabica coffee (*Coffea arabica*) in its Ethiopian center of origin
Matthias Beenhouwer (Heverlee/BE)

P82 Assessment of forest soil bacterial community structure and physiological characterization of key bacterial taxa
Salvador Lladó, Ivana Eichlerová, Věra Merhautová, Anna Davidová, Petr Baldrian (Prague/CZ)

P83 Isolation of phenol-catabolic genes by cultivation-independent functional screening from metagenome of soil artificially polluted by aromatic hydrocarbons
Hirofumi Nagayama, Tomonori Sugawara, Ryo Endo, Hiromi Kato, Yoshiyuki Ohtsubo (Sendai/JP)
Hiroshi Mori, Ken Kurokawa (Yokohama/JP), Yuji Nagata, Masataka Tsuda (Sendai/JP)

P84 Metagenomic analysis using long 16S amplicons and the Roche 454 GS FLX+ platform
Ovidiu Rücker, Alexandra Dangel, Stefan Kotschote (Martinsried/DE)

P85 Mining for bacterial chitinases – from a chitin-agar plate to the screening of metagenomes
Mariana Silvia Cretoiu (Groningen, Yerseke/NL), Jan Dirk van Elsas (Groningen/NL)

P86 Rice endophytes and their functions
Li-Sen Young, Meng-Wei Yeh (Huwei/TW)

P87 Co-occurrences of fungi and bacteria at biogeochemical interfaces in aged artificial soils
Annelie Steinbach, Julia Giebler, Florian Centler (Leipzig/DE), Stefanie Schulz, Geertje Pronk (Munich/DE)
Hauke Harms (Leipzig/DE), Michael Schloter (Munich/DE), Lukas Wick (Leipzig/DE)

P88 Phylogenetic analysis of the prokaryotic community in nitrogen-treated soils of a tropical montane mountain ecosystem in South Ecuador
Martin Engelhaupt (Göttingen/DE)

P89 Soil bacterial community succession along a salt marsh chronosequence – insights into temporal niche segregation promoting phylotypes co-existence
Francisco Dini-Andreote, Michele de Cássia Pereira e Silva (Groningen/NL), Xavier Triadó-Margarit
Emilio O. Casamayor (Blanes/ES), Jan Dirk van Elsas, Joana Falcão Salles (Groningen/NL)

P90 Dependence of soil bacterial community composition and diversity on land use types and management regimes
Kristin Kaiser (Göttingen/DE)

P91 Integrated bioinformatics analysis on the soil metagenome
Zhuofei Xu, Martin Hansen, Lars Hansen, Samuel Jacquiod, Søren Sørensen (Copenhagen/DK)

P92 Rhizosphere microbial community structure of different parental types of *Arabidopsis thaliana* MAGIC lines
Carla Porges, Tesfaye Wubet, François Busot (Halle a. d. S., Leipzig/DE)

P93 Response of AM fungal communities to land-use regimes of three biodiversity exploratories in Germany
Sandra Klemmer (Halle a. d. S., Leipzig/DE), Tesfaye Wubet (Halle/DE), Francois Buscot (Halle a. d. S., Leipzig/DE)

P94 Metagenomic approach to identify major cellulase families in agricultural soils under different management practices
Maria de Vries, Anne Schöler (Neuherberg/DE)

P95 Fungal community function in chronosequence of land abandonment
Emilia Hannula, Hans van Veen (Wageningen/NL)

P96 Phylogenetic and functional characterization of microbial communities in mesophilic compost soils harbouring different organic matters
Mingji Lu, Rolf Daniel, Silja Brady (Göttingen/DE)

P97 Recovery of soil microbial populations, function, and community composition following reclamation of a lignite surface mine
Terry Gentry, Justin Ng, Frank Hons (College Station, TX/US), Jizhong Zhou (Norman, OK/US)

P98 Structural and functional diversity of biodegradative populations in soil polluted by aromatics
Ondrej Uhlik, Jiri Wald, Michal Strejcek, Lucie Musilova, Tomas Macek (Prague/CZ)

P99 Metagenomic analysis uncovered novel structure of microbial communities involved in denitrification in paddy soil
Hideomi Itoh (Tokyo, Sapporo/JP), Keishi Senoo (Tokyo/JP), Satoshi Ishii (Tokyo, Sapporo/JP), Yutaka Shiratori (Nagaoka/JP)
Kenshiro Oshima (Kashiwa/JP), Shigeto Otsuka (Tokyo/JP), Masahira Hattori (Kashiwa/JP)

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Davide Bulgarelli (Dundee/GB, Cologne/DE), Paul Schulze-Lefert (Cologne/DE)

P101 Determining how oxygen legacy affects the trajectories of denitrifier function and structure in soil
Constance Roco, Joseph Yavitt, James Shapleigh (Ithaca, NY/US), Peter Dörsch, Lars Bakken, Åsa Frostegård (Ås/NO)

P102 A metagenomic study on the eubacterial populations in Alpine paleosols: Analysis based on 16S rRNA gene pyrosequencing and DGGE
Pentlavalli Prasanna, Chris Allen, Leonid Kulakov, Mike Larkin, Anna Kulakova, Alexandra Frau (Belfast/IE)

P103 Understanding the edaphic drivers of cellulose-degrading guilds in an austrian beech forest soil
Stephanie Eichorst, Florian Strasser (Vienna/AT), Tanja Woyke (Walnut Creek, CA/US), Dagmar Woebken (Vienna/AT)

P104 Soil microbial community structure and function based on N_2-fixation across different Agroecological Zones in New South Wales, Australia
Vanessa Pino, Rosalind Deaker, Mario Fajardo, Neil Wilson, Alex Mc Bratney (Sydney/AU)

P105 Role of bacteria-fungi interactions in hydrocarbon degradation studied in in situ microcosms
Paula Martinez-Lavanchy, Julia Giebler, Lukas Y. Wick (Leipzig/DE)

P106 The genetic diversity of archaeal ammonia oxidizers drives the potential nitrification rates in Dutch agricultural soils
Michele Pereira e Silva (Groningen/NL), Brigitte Schloter-Hai (Munich/DE), Franck Poly
Nadine Guilaumaud (Lyon/FR), Michael Schloter (Munich/DE), Jan Dirk van Elsas, Joana Falcão Salles (Groningen/NL)

P109 Performance of the universal primer pairs and different sequencing chemistries (Ion Torrent vs. 454) in the 16S rRNA based community analysis
Marja Tiirola (Jyväskylä/FI)

P110 Enrichment, isolation and characterization of microorganisms involved in reduction of crystalline iron(III) oxides in anoxic soil environments
Tomoyuki Hori, Tomo Aoyagi, Takashi Narihiro (Tsukuba/JP), Hideomi Itoh (Sapporo/JP), Atsushi Ogata
Satoshi Hanada, Yoichi Kamagata (Tsukuba/JP)

P112 Methanogenic communities in soils from the Tibetan Plataeu – insights from *mcr*A amplicon pyrosequencing
Sizhong Yang, Susanne Liebner, Mashal Alawi (Potsdam/DE), Corina Dörfer, Peter Kühn, Thomas Scholten (Tübingen/DE)
Dirk Wagner (Potsdam/DE)

P113 Characterization of the community structure and population dynamics of micropredators and pathogens in Wastewater Treatment Plants (WWTP)
Julia Johnke (Leipzig/DE), Edouard Jurkevitch, Zohar Pasternak, Yossi Cohen (Rehovot/IL), Antonis Chatzinotas (Leipzig/DE)

P114 Boom Clay Borehole Water, home of a diverse microbial community
Katinka Wouters, Hugo Moors, Patrick Boven, Natalie Leys (Mol/BE)

P115 Spatial and temporal variation of the fungal metagenome away from tree trunk of spruce and beech trees
Kezia Goldmann (Halle a. d. S., Leipzig/DE), François Buscot, Tesfaye Wubet (Halle a. d. S., Leipzig, Jena/DE)

P116 Unique functional diversity within Sphagnum peat bogs
Anastasia Bragina, Lisa Oberauner-Wappis, Bettina Halwachs, Gerhard G. Thallinger, Christian Berg, Henry Müller Gabriele Berg (Graz/AT)

P117 Phylogenetic and functional diversity of soil prokaryotic communities in temperate deciduous forests with different tree species
Amélie Dukunde, Rolf Daniel (Göttingen/DE)

P118 Brazilian microbiome project – revealing the unexplored microbial diversity – challenges and prospects
Victor Pylro, Daniel Morais (Viçosa/BR), Luiz Roesch (São Gabriel/BR), Alexandre Amaral (São Paulo/BR)
Penny Hirsch (Harpenden/GB), Marcos Tótola (Viçosa/BR)

P119 Metatranscriptomics reveals modification of the rhizosphere microbiome by crop plants
Mark Alston, Tom Turner, Philip Poole, David Swarbreck (Norwich/GB)

P120 Strategies for functional gene amplicon sequence processing
Michal Strejcek, Jiri Wald, Lucie Musilova, Tomas Macek, Ondrej Uhlik (Prague/CZ)

P121 Different responses of soil composition-driven microbial communities to plant litter and phenanthrene in artificial soils
Doreen Babin (Braunschweig/DE), Cordula Vogel (Munich, Freising-Weihenstephan/DE), Sebastian Zühlke
Michael Spiteller (Dortmund/DE), Ingrid Kögel-Knabner (Munich, Freising-Weihenstephan, Garching/DE)
Kornelia Smalla (Braunschweig/DE)

P122 A cross-disciplinary soil-proteomics approach for predicting switches between hydrophilic and hydrophobic soil surface responses
Gerry Quinn, Elizabeth Bond, Ed Dudley (Swansea/GB), Peter Mathews (Plymouth/GB), Stefan Doerr
Geertje Van Keulen (Swansea/GB)

P123 Protein-stable isotope probing (protein-SIP) identified *Pseudomonadales* and *Xanthomonadales* as active bacterial groups during leaf litter degradation in tobacco soil
Nico Jehmlich, René Kermer, Tesfaye Wubet, François Buscot (Leipzig/DE), Jana Seifert (Stuttgart/DE)
Martin von Bergen (Leipzig/DE; Aalborg/DK)

P124 Population genomics of the legume plant host specific selection of rhizobial genotypes
Beatriz Jorrín, Juan Imperial (Pozuelo de Alarcón/ES)

P125 Soil microbial community structure and function in relation to water regime changes in the Namib Desert
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P126 Soil environment as a source of new tools for biotransformation and bioremediation
Ewa Furmanczyk, Paweł Krawczyk, Dorota Adamska, Maciej Sojka, Grzegorz Spólnik, Adam Sobczak, Leszek Lipiński
Andrzej Dziembowski (Warsaw/PL)

P127 Parallel diversity assessment of rhizosphere and endophytic communities
Lucie Musilova, Michal Strejcek, Martina Novakova (Prague/CZ), Elizabeth Pilon-Smits (Fort Collins, CO/US)
Tomas Macek, Ondrej Uhlik (Prague/CZ)

P128 Soil microbial community structure vs. function – Who's driving ?
Sebastien Cecillon, Timothy M. Vogel (Ecully/FR)

P129 Effect of wetland vegetation, water level and fertilization on bacterial and fungal community composition and activity: mesocosm manipulative experiment
Jiri Barta, Tomas Picek, Eva Kastovska, Hana Santruckova (Ceske Budejovice/CZ), Sarah Owens (Lemont, IL/US)
Keith Edwards (Ceske Budejovice/CZ)

P130 Changes in bacterial and archaeal community composition in mineral soils during long-term ecosystem development
Stephanie Turner, Marco Blöthe, Robert Mikutta, Sandra Meyer-Stüve, Reiner Dohrmann, Georg Guggenberger
Axel Schippers (Hannover/DE)

P131 Diversity of soil crust prokaryotic microbiota and its response to physical disturbance
Stefanie Maier, Ines Aline Aschenbrenner (Graz/AT), Sebastian Schmidt (Zurich/CH), Martin Grube (Graz/AT)

P132 Genetic and functional diversity of soil microbial communities associated to grapevine plants and wine quality
Stefano Mocali, Arturo Fabiani (Firenze/IT), Eiko Kuramae, Mattias de Hollander (Wageningen/NL)
George Kowalchuk (Wageningen, Amsterdam, Utrecht/NL), Nadia Vignozzi, Giuseppe Valboa, Roberta Pastorelli (Firenze/IT)
Flavio Fornasier (Gorizia/IT), Edoardo Costantini (Firenze/IT)

P133 How do warming and grazing affect soil bacterial diversity in the Mongolian steppe?
Aurora MacRae-Crerar, Pierre Liancourt, Laura Spence (Philadelphia, PA/US)
Bazartseren Boldgiv (Philadelphia, PA/US; Ulaanbaatar/MN), Daniel Song (Philadelphia, PA/US)
Jack Gilbert, Sarah Owens (Argonne/FR; Chicago, IL/US), Jarrad Hampton-Marcell (Argonne/FR)
Brendan Hodkinson, Brenda Casper, Peter Petraitis (Philadelphia, PA/US)

P135 Bacterial diversity in the rhizosphere of maize – scaling potential effects of a genetic modification to biogeographic variables
Astrid Näther, Christoph C. Tebbe (Braunschweig/DE)

P136 Phages as vectors and indicators of biological information in the Earth's Critical Zone
Anja Narr, Lukas Wick, Hauke Harms, Antonis Chatzinotas (Leipzig/DE)

P137 Soil particle size fractions harbour microbial communities with different metabolic potentials and activities to degrade phenol
Michael Hemkemeyer (Braunschweig/DE), Bent T. Christensen (Foulum/DK), Rainer Martens
Christoph C. Tebbe (Braunschweig/DE)

P138 Variations in the structure and functional activity of soil microbial communities associated with phase of crop rotation and annual rice cycle
Ana Lopes (Porto/PT), Diana Bello, Ángeles Prieto-Fernández, Carmen Trasar-Cepeda, Fernando Gil-Sotres
Maria Leirós (Santiago de Compostela/ES), Célia Manaia, Olga Nunes (Porto/PT)

P139 High elevation grasslands dominated by *Carex curvula* and *Nardus stricta* select for similar fungal communities in the Alps and in the Carpathians.
Roberto Geremia (Grenoble/FR), Mihai Puscas (Cluj-Napoca/RO), Jean-Marc Bonneville (Grenoble/FR)
Lucie Zinger (Toulouse/FR), Philippe Choler (Grenoble/FR)

P140 Plant species and soil type affect rhizosphere microbial community composition
Susanne Schreiter, Ute Zimmerling (Braunschweig/DE), Petra Zocher (Großbeeren/DE), Guo-chun Ding (Braunschweig/DE)
Rita Grosch (Großbeeren/DE), Kornelia Smalla (Braunschweig/DE)

P141 Responses of tundra soil bacterial communities to increased nitrogen availability
Lars Ganzert, Minna Männistö, Sari Stark (Rovaniemi/FI), Marja Tiirola, Sukithar Rajan (Jyväskylä/FI)
Max Häggblom (New Brunswick/CA)

P142 Taxonomic and functional profiling of the microbial community from a thermophilic production-scale biogas plant by a metagenome approach
Irena Maus (Bielefeld/DE)

P143 Microbial communities of different dimensional groups of soil aggregates under the extreme agricultural systems
Ekaterina Ivanova, Olga Kutovaya, Azida Thakahova, Evgeny Andronov (Moscow/RU)

P144 Seasonal dynamics of plant associated bacterial communities in low arctic fell tundra
Riitta Nissinen (Jyväskylä/FI; Groningen/NL), Minna Männistö (Rovaniemi/FI), Anna Kielak-Butterbach (Wageningen/NL)
Jan Dirk van Elsas (Groningen/NL)

P145 Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating wet and dry fermentation
Yvonne Stolze (Bielefeld/DE)

P146 Changes in soil microbial community structure under influence of leaching compounds from radioactive oily waste: a column experiment
Polina Galitskaya (Kazan/RU), Stefan Ratering, Sylvia Schnell (Giessen/DE), Svetlana Selivanovskaya (Kazan/RU)

P147 Detection and characterization of subsurface life in the Iberian Pyritic Belt (IPB)
Alejandro Arce-Rodríguez (Braunschweig/DE), Fernando Puente-Sánchez, Miriam García-Villadangos
Victor Parro, Ricardo Amils (Madrid/ES), Kenneth N. Timmis (Braunschweig/DE)

P148 The dark side of the metagenomes
Antonio Fernàndez-Guerra, Renzo Kottmann (Bremen/DE), Albert Barberán Torrents (Boulder, CO/US)
Frank Oliver Glöckner (Bremen/DE), Emilio O. Casamayor (Blanes/ES)

P149 Adaptation of soil microbial community function and structure to chronic metal pollution
Anders Lanzén (Neiker-Tecnalia/ES)

Biocontrol

P150 Metagenomics and metatranscriptomics of natural disease suppressive soils
Ruth Gómez Expósito, Allison Jack, Irene de Bruijn, Emilie Chapelle, Joeke Postma, Jos Raaijmakers (Wageningen/NL)

P151 The microbiome of medicinal plants and its potential for biocontrol and promotion of plant growth and quality
Martina Köberl, Ruth Schmidt (Graz/AT), Elshahat M. Ramadan (Cairo/EG), Henry Müller, Anastasia Bragina (Graz/AT)
Kornelia Smalla (Braunschweig/DE), Gabriele Berg (Graz/AT)

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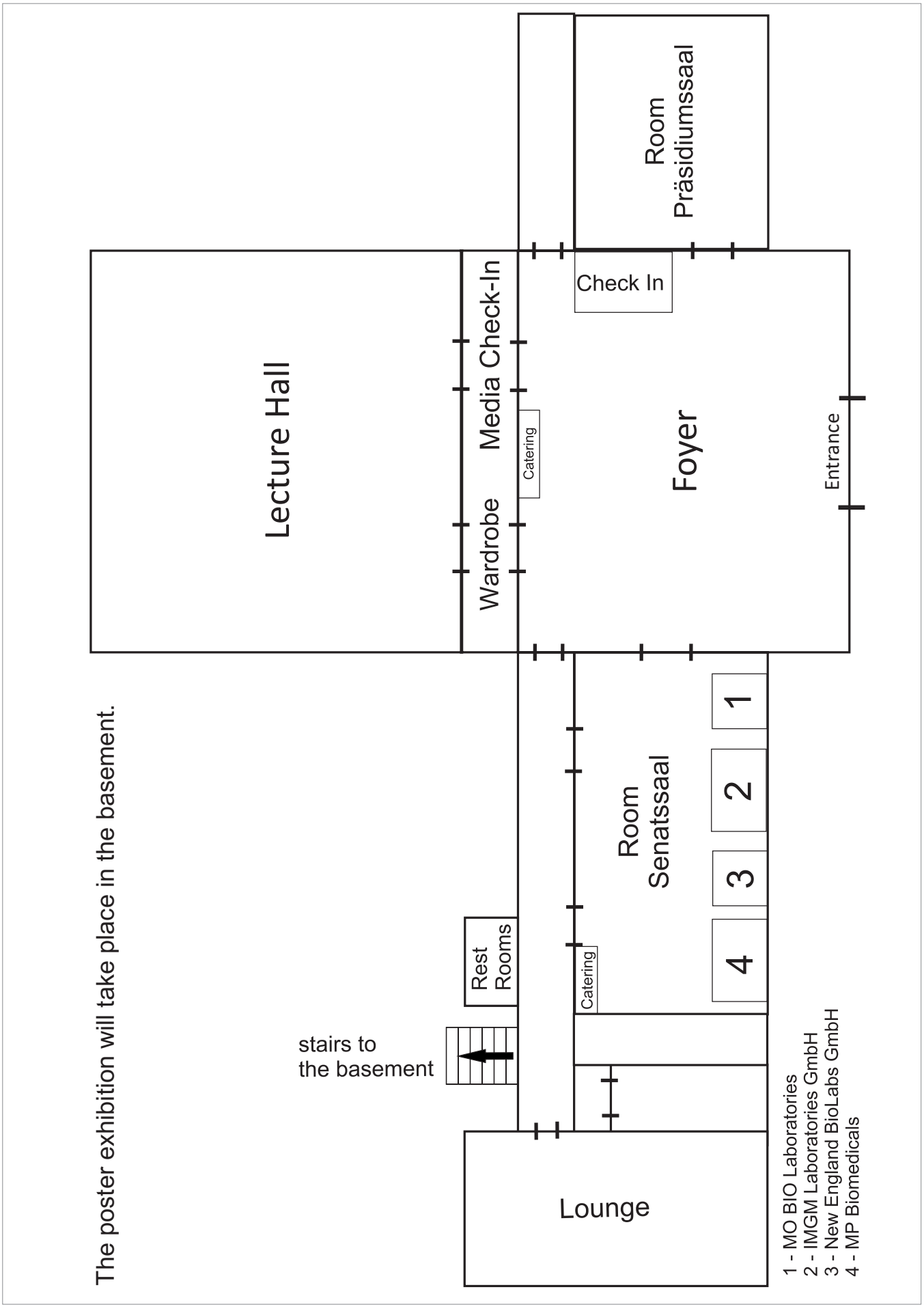

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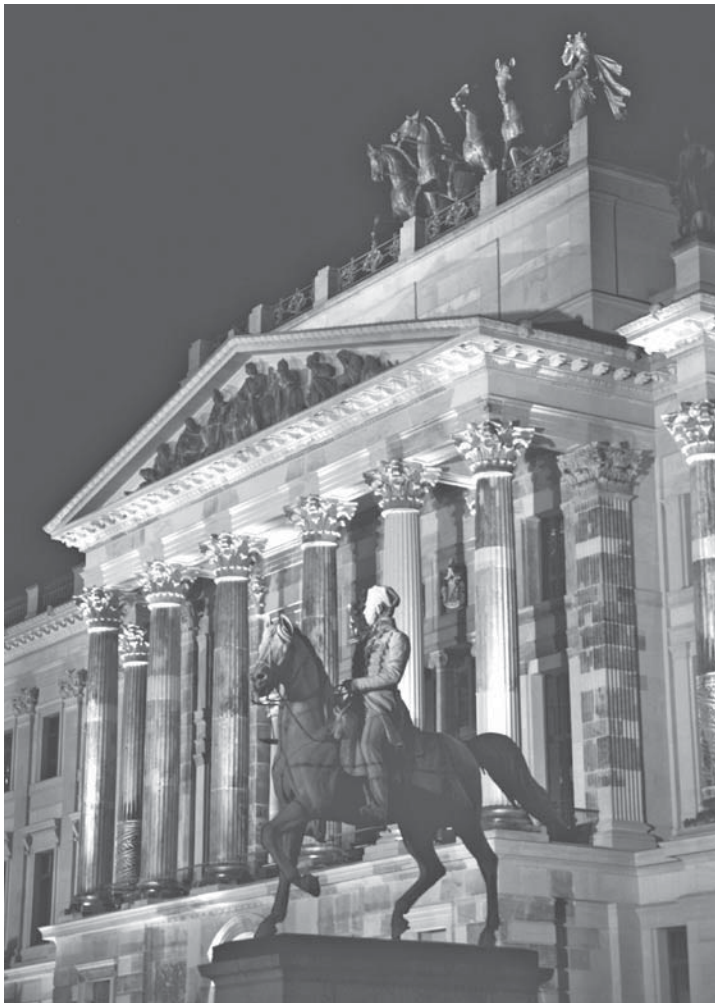
Conference Dinner

We are more than happy to invite all participants to join us for our conference buffet on Thursday. This social event is also taking place at the Thünen Forum and will start with a bright surprise just outside the Forum. We would like to encourage our young scientists to approach the more experienced generation, to network and just enjoy this great Metagenomics community.

Date	Thursday, 12 December 2013
Begin	19^{15} hrs.
Price	included
Shuttle	Busses back to the hotels leave at 21^{30} hrs. in front of the Forum



Registration Fees

Workshop (11 & 13 December)	45 EUR
Regular	345 EUR
Conference Buffet	included
Accompanying Person Conference Buffet	45 EUR

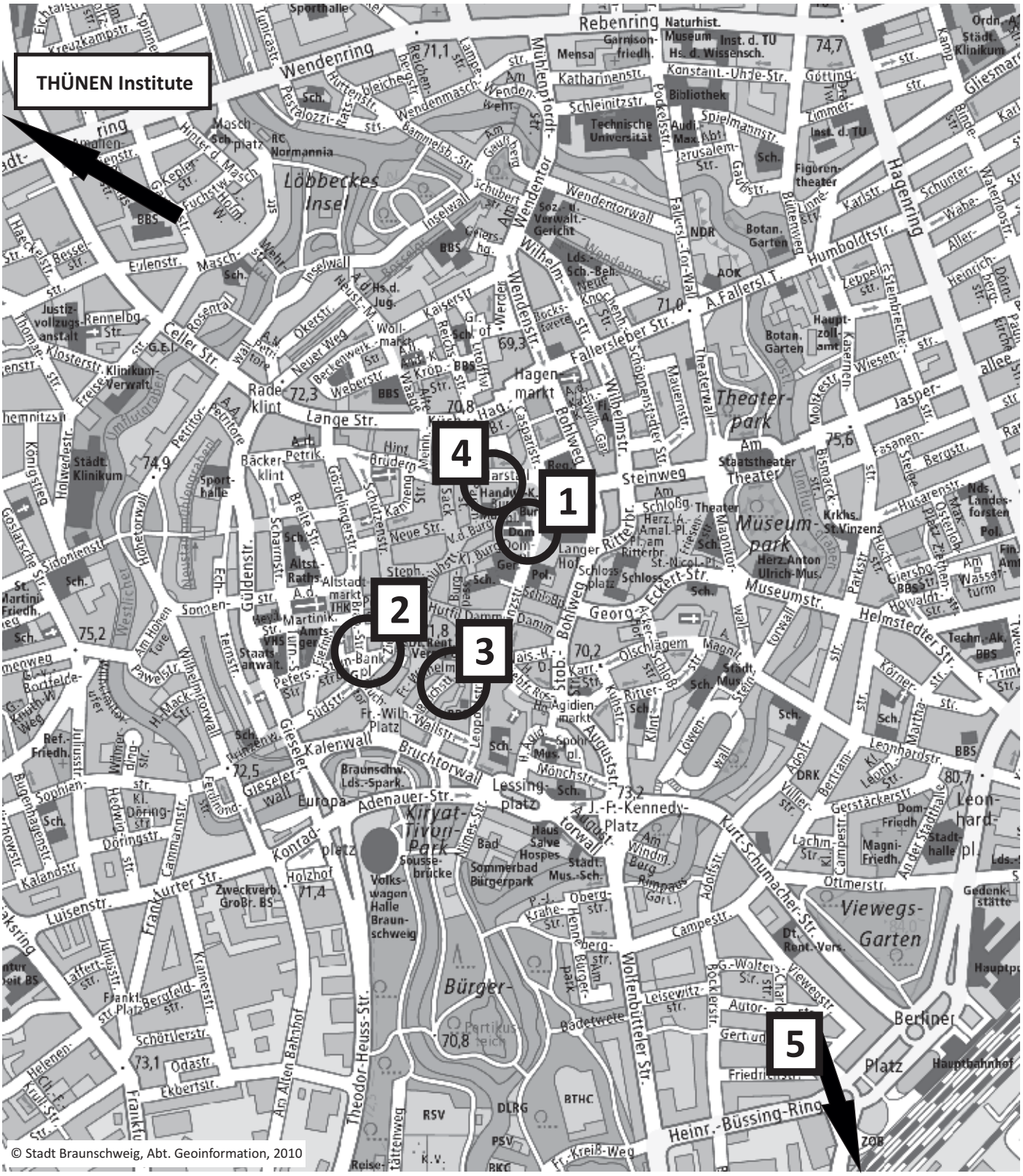
Public Transportation to the venue

Braunschweig Central Station:
Taking bus line 461 or 411, please exit at "Bundesallee" (about 15 minutes walking distance from the Thünen Forum).
Hotel Arcadia: At the bus stop "Staatliche Untersuchungsämter" please take bus line 411, heading to "Lamme", and exit at "Bundesallee" (about 15 minutes walking distance from the Thünen Forum).
Hotel Deutsches Haus: At the bus stop "Rathaus" board either bus line 560 or 411 and exit at "Bundesallee" (about 15 minutes walking distance from the Thünen Forum).
Hotel Frühlings-Hotel and Best Western City-Hotel Braunschweig: At bus stop "Friedrich-Wilhelmplatz" take bus line 461 (heading to "Lamme" or "PTB") and exit at "Bundesallee" (about 15 minutes walking distance from the Thünen Forum).

Shuttle Service from hotels to Thünen Institute

11 December, 2013	07:25 dep. Arcadia Hotel • 07:35 dep. Frühlings-Hotel 07:40 dep. Best Western City Hotel • 07:45 dep. Deutsches Haus 08:00 arr. Thünen Institute
	11:55 dep. Arcadia Hotel • 12:05 dep. Frühlings-Hotel 12:10 dep. Best Western City Hotel • 12:15 dep. Deutsches Haus 12:30 arr. Thünen Institute
	19:00 dep. Thünen Institute • 19:15 arr. Deutsches Haus (Christmas Market) 19:20 arr. Best Western City Hotel • 19:25 arr. Frühlings-Hotel 19:35 arr. Arcadia Hotel
12 December, 2013	07:55 dep. Arcadia Hotel • 08:05 dep. Frühlings-Hotel 08:10 dep. Best Western City Hotel • 08:15 dep. Deutsches Haus 08:30 arr. Thünen Institute
	21:30 dep. Thünen Institute • 21:45 arr. Deutsches Haus 21:50 arr. Best Western City Hotel • 21:55 arr. Frühlings-Hotel 22:05 arr. Arcadia Hotel
13 December, 2013	08:10 dep. Arcadia Hotel • 08:20 dep. Frühlings-Hotel 08:25 dep. Best Western City Hotel • 08:30 dep. Deutsches Haus 08:45 arr. Thünen Institute
	14:00 dep. Thünen Institute • 14:15 arr. Deutsches Haus 14:20 arr. Best Western City Hotel • 14:25 arr. Frühlings-Hotel 14:30 arr. Main Train Station • 14:40 arr. Arcadia Hotel
	17:45 dep. Thünen Institute • 18:00 arr. Deutsches Haus 18:05 arr. Best Western City Hotel • 18:10 arr. Frühlings-Hotel 18:15 arr. Main Train Station • 18:25 arr. Arcadia Hotel

City Map



1 Christmas Market • Burgplatz

2 Frühlings-Hotel • Bankplatz 7

3 Best Western City Hotel-Braunschweig • Friedrich-Wilhelm-Straße 26

4 Deutsches Haus • Ruhfäuchtenplatz 1

5 Arcadia Hotel PlayOff Braunschweig • Salzdahlnumer Straße 137

Hanover Airport Shuttle
For your return trip from Braunschweig to Hanover airport it is possible to book a private shuttle service at extra charge.
In case you are interested, please contact and quote „Soil Metagenomics" as reference:

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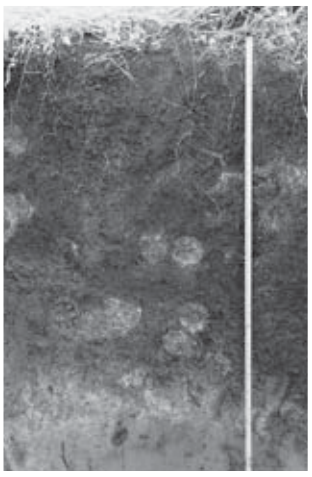

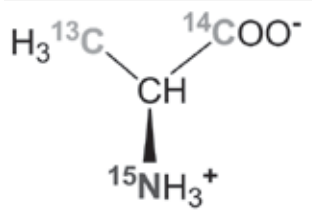

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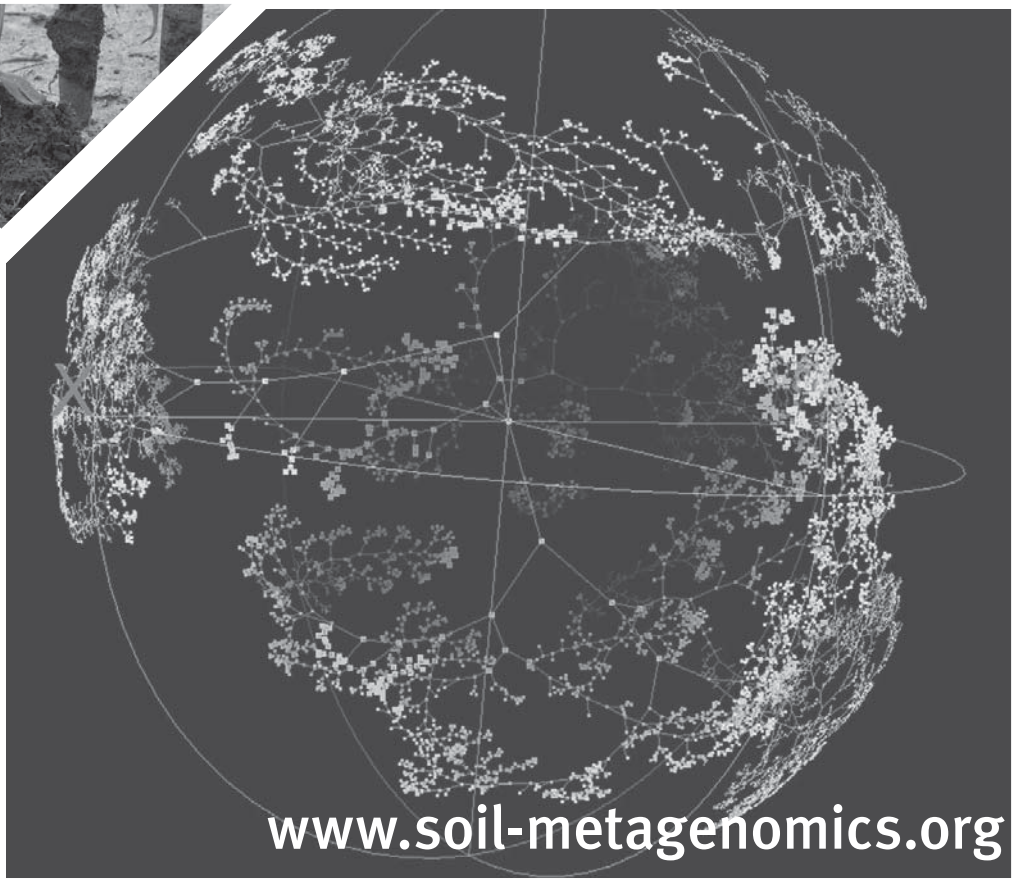
20 July 2011

2nd THÜNEN SYMPOSIUM ON SOIL METAGENOMICS

MINING AND LEARNING FROM METAGENOMES

plus Workshop on Bioinformatic Tools

ABSTRACTS



THÜNEN

www.soil-metagenomics.org

KN1
Environmental Bioinformatics - with a focus on "Marine"

F. O. Glöckner[1]
[1]Max Planck Institute for Marine Microbiology and Jacobs University Bremen gGmbH, Bremen, Germany

Investigations in molecular biology have transitioned from single experiments to high-throughput endeavours spearheaded by genomic science. Although the genomic revolution is rooted in medicine and biotechnology, environmental studies, most notably those of marine ecosystems, currently deliver the highest quantity of data. New sequencing technologies are providing an increasingly powerful resource to investigate microbial diversity and function at the gene level.

The talk will elaborate on our approaches to transform the wealth of sequence and contextual (meta)data into biological knowledge. We are currently developing components (SILVA (www.arb-silva.de)), MEGX.net (www.megx.net)) and standards (MIxS (www.gensc.org)) for an integrative bioinformatic workbench to bring together organism diversity and abundance data, functional data (genomic data), and the environmental data contextualising them. This workbench will allow the persistent and dynamic study of relationships between organisms, their genomic repertoire, and their environment.

The talk will further outline the goals and perspectives of the EU 7FP "Ocean of Tomorrow Project" Micro B3 (Biodiversity, Bioinformatics, Biotechnology,www.microb3.eu) as well as the Ocean Sampling Day initiative (OSD,www.oceansamplingday.org). OSD is scheduled to take place worldwide on summer solstice - 21 June 2014, with pilots conducted in 2012 and 13. The cumulative samples, fixed in time and space supplemented with a broad set of geo-referenced environmental parameters, will contribute to determine a baseline of marine biodiversity and functions on the molecular level.

KN2
Current Bioinformatic challenges in soil shotgun Metagenomics

F. Meyer[1]
[1]Argonne National LaboratoryArgonne National Laboratory, Argonne, United States

The analysis of shotgun metagenomic sequencing data from soil is one of the most challenging and computationally intensive fields in Biology at this time. While sequencing cost plummet, computational rise significantly with no end in sight. The method uncertainty makes the rational design of studies very difficult.

I will highlight some of the known pitfalls associated with shotgun metagenomic sequence analysis, including the notions of sequence quality, metagenomic sequence assembly and prediction of genes from metagenomic data.

KN3
Fungal communities in soils - structure, dynamics and functioning

P. Baldrian[1]
[1]Institute of Microbiology of the ASCR, Laboratory of Environmental Microbiology, Praha 4, Czech Republic

Fungi are important drivers of environmental processes as decomposers, symbionts of plants or pathogens. Although their communities are less diverse than bacterial ones - approximately by one order of magnitude - they are still highly diverse with several hundreds of taxa in a single gram of soil. Due to larger size and greater dispersal limitations, fungal communities are more diverse across space than bacterial ones and they are largely shaped by the dominant plant taxa. Fungal communities are dynamic across time: along a succession series of dead plant biomass as well as throughout the changing seasons of the year, both the structure and total abundance of fungi profoundly changes: while the plant symbionts are most affected by seasonal changes of photosynthetic primary production, saprotrophic fungi reflect the availability and quality of litter or organic matter in soil. Fungi were demonstrated to act as the key decomposers in terrestrial ecosystems due to their ability to produce enzymes decomposing the complex compounds of dead plant biomass such as lignin, cellulose and hemicellulose. The decomposition is a concerted action of tens to hundreds of taxa that possess the genes required for enzyme production. Although the recent advances in molecular biology give us the tools to analyze large amount of sequences, the understanding of fungal community composition is still limited due to the unclear relationships between fungal biomass, genome sizes and rDNA abundance. Much effort should be thus still invested in the exploration of alternative molecular markers.

KN4
Metagenomics of the rhizosphere

J. M. Tiedje[1], A. Garoutte[1], J. Guo[1], J. Fish[1], A. Howe[1], B. Zhang[1], C. Xue[1], Q. Wang[1], C. T. Brown [1], J. Cole[1]
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Metagenomics and its namesake, the microbiome, has become a core of new age microbial ecology. Metagenomics is still in its infancy, but now beginning to provide some real returns on investment. But, it has many faces - those determined by the various methods employed, the complexity of the different communities and the resources available. We have investigate none of the most challenging, the rhizosphere of the three major U.S. biofuel crops, maize, switchgrass and *Miscanthus*, with the goal of exploring what the rhizospheres of these crops select and whether features beneficial to lower cost, sustainable production can be identified and managed for benefit. We have explored the metatranscriptome of the “close” *Miscanthus* rhizosphere obtaining 205 million reads, and analyzed both the unassembled and assembled reads, and against three data sets, MG_RAST, CAZy (carbohydrate) and our curated set of soil-specific reference genomes. The most abundant expressed proteins included sequences related to RNA and protein metabolism, phage-related proteins and some unannotated genes that are highly expressed. The assembled transcripts provide increased annotation and more confidence in the annotation. For the metagenomics phase, we sampled seven replicates that are each composites of several rhizospheres of the three crops and have sequenced each replicate by 1 lane of Illumina, yielding 1 Tb of data. The data are analyzed by our scalable metagenome assembly, gene-targeted assembly of *nifH* using our Xander tool, which uses a Hidden Markov Model to guide local assembly, and data mining of the shotgun short reads using reference gene models, e.g. rRNA. We have also used amplicon sequencing of rRNA and *nifH* genes for comparative work on a larger number of samples and to achieve greater depth for the target gene.

KN5
Reconstruction of microbial nutrient cycles in soil using Metagenomic approaches

S. Schulz[1], A. Bannert[1], M. de Vries[1], A. Schöler[1], J. Ollivier[1], M. Granitsiotis[1], M. Engel[1], M. Schloter[1]
[1]Helmholtz Zentrum München, Research Unit Environmental Genomics, Neuherberg, Germany

Microbial communities 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. Consequently, a lot of important processes or microbial taxa might have been overlooked. 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 generation sequencing techniques, allows an unbiased view on the community structure and function of ecosystems.

Thus 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*. Here, we want to demonstrate the power of this approach showing several examples.

An experiment with rice plants, which were cultivated in differently aged paddy soils and sampled at the tillering and flowering stage, revealed significant differences regarding the carbohydrate metabolism based on SEED and KEGG classification systems. Moreover, the reconstruction of the whole nitrogen cycle revealed that previously ignored processes like nitrite ammonification and nitroalkane oxidation predominate in paddy soils.

In another study a multivariate comparison of metagenomic data from permafrost soils of the Tibetan Plateau with soils from arctic environments, temperate grasslands and tropical forests, revealed not surprisingly a strong separation of the microbial communities from permafrost environments compared to the other ecosystems based on differences in carbon and nitrogen turnover. However we could nicely demonstrate that, based on different redox conditions present in the permafrost soils from the Tibetan Plateau (dryer conditions at the permafrost interface), the role of methanogenesis was lower and the potential for other redox processes mainly iron reduction and denitrification was more pronounced than in waterlogged soils from other permafrost environments.

Altogether, our examples illustrate the power of metagenomic data to reconstruct nutrient cycles in soil ecosystems, there are also 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*).

Key words: Soil metagenomics, permafrost soil, paddy soil, tillage, carbon and nitrogen cycle

KN6
A cross-site investigation of belowground community responses to shifts in nutrient availability

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[2]University of Colorado at Boulder, Department of Ecology and Evolutionary Biology, Boulder, United States

Elevated nutrient inputs to terrestrial ecosystems can have profound effects on a broad range of soil microbial processes including those processes that are the key determinants of soil carbon (C) sequestration rates. Previous work has demonstrated that nitrogen (N) additions often decrease microbial biomass and respiration rates with associated shifts in bacterial community structure, suggesting that these shifts may be responsible for nutrient-driven decreases in soil C fluxes. In addition, through marker gene sequencing, we recently confirmed that elevated nutrient inputs to soils elicit substantial changes in both bacterial and fungal community composition across a wide array of grassland sites from across the globe. To better understand how these shifts in community composition relate to alterations of microbial function, we performed shotgun metagenomic sequencing on samples from replicated control and nutrient amendment plots from six of the grassland sites (72 samples in total). These data demonstrated that the functional attributes of the microbial communities differed most strongly between sites, but N and P amendments also had significant impacts on the functional attributes of the belowground communities within sites. These results suggest strong concordance between shifts in soil microbial community composition and function with elevated nutrient inputs. Moreover, the results are consistent with the hypothesis that alterations of soil microbial community structure may be a key mechanism driving changes in soil C sequestration under elevated nutrient deposition.

KN7
Turnover of soil microbial diversity is driven by wide-scale environmental heterogeneity

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[1]UMR 1347 Agroécologie, Dijon, France
[2]Platform Genosol, UMR 1347 Agroécologie, Dijon, France

Spatial scaling and determinism of the wide-scale distribution of macroorganisms' diversity has been largely demonstrated over a century. For microorganisms, this fundamental question requires more thorough investigation. Here, we investigate the spatial structuration of soil microbial communities on a wide scale, particularly bacteria and fungi, and focus on both processes and filters shaping their abundance and diversity. Soil bacterial and fungal communities were characterized on the largest spatially explicit soil sampling available in France (2,085 soils over *ca.* 5.3 10^5 km^2) by using molecular techniques (biomass, fingerprinting and pyrosequencing of ribosomal genes).

With this framework, we provide an extensive map of soil molecular microbial biomass, revealing its structuration into non-random spatial patterns determined by local factors (soil pH, organic carbon, texture, and land use) rather than by global filters (e.g. climate). By applying the taxa-area relationship and developing an innovative evaluation of the habitat-area relationship, we show that the turnover rate of bacterial diversity in soils on a wide scale is highly significant and strongly correlated with the turnover rate of soil habitat. This result highlights the importance of environmental selection in shaping soil microbial diversity. In addition, by simulating new landscape configurations, we suggest that dispersal of soil microbes may be limited, in agreement with Hubbell's Neutral Theory. Variance partitioning approaches allowed the identification of the environmental filters shaping both bacterial and fungal diversity: pH, trophic resources (organic carbon, nitrogen and C:N ratio), texture and land use; and of the scales at which dispersal was limited: coarse (80 - 110 km) and medium (40 - 60 km) spatial scales. Applying next generation sequencing techniques on this framework supports these results and show that soil microbial diversity turnover is much higher than suggested in previous studies.

Consequently, as the diversity of micro- and macroorganisms appears to be driven by similar processes (dispersal and selection), maintaining diverse and spatially structured habitats is essential for soil biological patrimony and the resulting ecosystem services.

KN8
Soil microbial community responses to warming as revealed by Metagenomics.

K. Konstantinidis[1], C. S. Wilder[1]
[1]Georgia Institute of Technology, School of Civil & Environmental Engineering and School of Biology (Adjunct), Atlanta, United States

To provide insights into how soil microbial communities acclimate to warming, we analyzed twelve whole-community shotgun metagenomic datasets from a grassland soil in Midwest USA (Oklahoma); half representing soil that was undergoing infrared warming by 2°C for 10 years, which simulated the effects of climate change, and the other half representing the adjacent soil that received no warming and thus, served as control. Our analyses revealed that heated communities showed small but significant shifts in composition and metabolic potential and these shifts were community-wide as opposed to being attributable to a few taxa. Key metabolic pathways related to carbon cycling, e.g., cellulose degradation (~13%) and CO_2 emissions (~10%), and the nitrogen cycle, e.g., denitrification (~12%), were significantly enriched in heated communities, which were associated, at least in part, with higher primary productivity of the aboveground plant communities stimulated by warming. These findings were also reflected in companion metatranscriptomics data and physicochemical measurements of soil organic carbon content and respiration rate, revealing that most of the additional, plant-derived soil carbon under warming was likely respired by microbial activity. Warming also enriched for a higher abundance of sporulation genes and genomes with higher G+C% content. Collectively, our results indicate that the microbial communities of the temperate grassland soils play important roles in mediating the feedback responses to climate change and advance understanding of the modes and tempo of community adaptation to environmental perturbations. We will also report on the bioinformatic pipelines that enabled the above analyses as well as how our findings from temperate soils compared to the findings from similar experimental warming in Alaskan permafrost soils.

KN9

Multi-substrate isotope labeling and metagenomic analysis of active soil bacterial communities

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Soil microbial diversity represents the largest global reservoir for the discovery of novel microorganisms and enzymes. Both stable-isotope probing (SIP) and metagenomics have been used to access uncultured microbial diversity, but few studies have combined these two methods for accessing the biotechnological potential of soil genetic diversity, and fewer yet have employed functional metagenomics for recovering novel genes and enzymes for bioenergy or bioproduct applications. In this study, we demonstrate the power of combining functional metagenomics and SIP using multiple plant-derived carbon substrates and diverse soils for characterizing active soil bacterial communities and recovering glycosyl hydrolases based on gene expression. We incubated three disparate Canadian soils (tundra, temperate rainforest and agricultural) with five native carbon (^{12}C) or stable-isotope labelled (^{13}C) carbohydrates (glucose, cellobiose, xylose, arabinose, and cellulose). Sampling at defined time intervals (one, three, and six weeks) was followed by DNA extraction and cesium chloride density gradient ultracentrifugation. Denaturing gradient gel electrophoresis (DGGE) of all gradient fractions confirmed the recovery of labeled nucleic acids. Sequencing of original soil samples and labeled DNA fractions demonstrated unique heavy DNA patterns associated with all soils and substrates. Indicator species analysis revealed an important role for many uncultured and unclassified bacterial taxa in the heavy DNA for all soils and substrates. Among characterized taxa, *Pseudomonadales*, *Actinomycetales*, *Rhizobiales*, *Xanthomonadales*, *Sphingomonadales*, and *Burkholderiales* were among the bacterial "indicator species" for the heavy substrates and soils tested. Annotated metagenomic data suggested diverse glycosyl hydrolase gene representation within the pooled heavy DNA. By screening only 3000 inserts derived from the ^{13}C-cellulose heavy DNA, we demonstrate the power of stable-isotope probing and functional screens by recovering six clones with activity against carboxymethylcellulose and methylumbelliferone-based substrates.

KN10

Metamoanics, lost watches and glimmers of hope

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'Traditional' molecular ecology techniques have now been used for 20 years to characterise natural microbial communities. Early studies were largely descriptive but have been complemented, but not replaced, by those aimed at determining whether links exist between community composition, environmental characteristics and environmental factors and attempts to determine mechanisms driving microbial community composition. They also activated or generated interest in broader ecological questions and theories, and their relevance to microbial diversity, activity and ecosystem function, and awakened long-standing debates, such as those surrounding bacterial and archaeal species concepts. Development of this process has continued with the availability of high-throughput sequencing of phylogenetic and functional marker genes. This potentially provides useful information, but studies often apply new techniques without determining critically which techniques are required for specific ecological questions. Soil metagenomics, metatranscriptomics and other omics approaches introduce something different and something new by providing an holistic view of gene or transcript content, independent of environmental structure or even cellular structure. While emphasis on technical and bioinformatics challenges persists, the greatest challenges are arguably conceptual, in determining which, if any, fundamental ecological questions can be addressed using omics approaches; whether new concepts are required to direct meaningful experimental studies; and the extent to which knowledge of soil microbial ecology gained during the past 130 years can inform such conceptual challenges.

KN11
Trends and barriers for plasmid- and phage-mediated lateral gene transfer

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Gene acquisition by lateral gene transfer (LGT) is an important mechanism for natural variation among prokaryotes. Laboratory experiments show that protein-coding genes can be laterally transferred extremely fast among microbial cells, inherited to most of their descendants, and adapt to a new regulatory regime within a short time. Recent advance in the phylogenetic analysis of microbial genomes using networks approach reveals a substantial impact of LGT during microbial genome evolution. Phylogenomic networks of LGT among prokaryotes reconstructed from completely sequenced genomes uncover barriers to LGT in multiple levels including (i) barriers to gene acquisition in nature including physical barriers for gene transfer between cells, (ii) genomic barriers for the integration of acquired DNA, and (iii) functional barriers for the acquisition of new genes.

KN12
Horizontal gene transfer of short DNA sequences

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Horizontal gene transfer (HGT) is a driving factor in short term adaptation and long term evolution of bacterial populations. Our research group focuses on improving the overall understanding of HGT processes in bacterial communities (www.uit.no/forskning/mmpe). We seek to identify key factors that regulate recombination between genomes through experimentation and prediction. Bacterial recombination is studied at the molecular, cellular and population scale levels. Population scale studies offer the ability to quantify host fitness effects of specific recombination events and thereby, through mathematical modelling, predict the fate of particular HGT events in larger populations over time.

Here, I present some of our recent studies that expand the understanding of the range of DNA substrates available for natural transformation in bacteria. The presentation will focus on the transformation potential of short DNA fragments including the effects of chemical damages. A new non-RecA dependent mechanism for natural acquisition of DNA fragments by bacteria is presented.

Domingues, S., Harms, K., Fricke F.W. P.J. Johnsen, G. da Silva, and K. M. Nielsen. 2012. Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species. *PLoS Pathogens* 8(8): e1002837.

Starikova, I., Harms, K., Lunde, T. T. M, Haugen, P., Primicerio, R., Samuelsen, Ø., Nielsen, K. M., and Johnsen, P. J. 2012. A fitness trade-off between gene cassette capture and stability of integrons. *PLoS Pathogens* 8(11): e1003043.

Overballe-Petersen *et al*. Natural transformation by degraded DNA allows for bacterial genetic exchange across geological time. *PNAS. (in press).*

KN13

Combination of conceptual and technical approaches for exploiting soil microbial ressources.

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The soil microorganisms are responsible for a range of critical functions including those that directly affect our quality of life (e.g., antibiotic production and resistance – human and animal health, nitrogen fixation -agriculture, pollutant degradation – environmental bioremediation). Nevertheless, genome structure information has been restricted by a large extent to a small fraction of cultivated species. This limitation can be circumvented now by modern alternative approaches including metagenomics or single cell genomics. Metagenomics includes the data treatment of DNA sequences from many members of the microbial community, in order to either extract a specific microorganism's genome sequence or to evaluate the community function based on the relative quantities of different gene families. In my talk I will show how these metagenomic datasets can be used to estimate and compare the functional potential of microbial communities from various environments with a special focus on antibiotic resistance genes. However, metagenomic datasets can also in some cases be partially assembled into longer sequences representing microbial genetic structures for trying to correlate different functions to their co-location on the same genetic structure. I will show how the microbial community composition of a natural grassland soil characterized by extremely high microbial diversity could be managed for sequentially attempt to reconstruct some bacterial genomes.

Metagenomics can also be used to exploit the genetic potential of environmental microorganisms. I will present an integrative approach coupling *rrs* phylochip and high throughput shotgun sequencing to investigate the shift in bacterial community structure and functions after incubation with chitin. In a second step, these functions of potential industrial interest can be discovered by using hybridization of soil metagenomic DNA clones spotted on high density membranes by a mix of oligonucleotide probes designed to target genes encoding for these enzymes. After affiliation of the positive hybridizing spots to the corresponding clones in the metagenomic library the inserts are sequenced, DNA assembled and annotated leading to identify new coding DNA sequences related to genes of interest with a good coverage but a low similarity against closest hits in the databases confirming novelty of the detected and cloned genes.

KN14

Mining large-insert soil metagenomic libraries for antimicrobial activity and biosynthetic pathways

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Introduction

Many clinically-used antibiotics are derived from cultured soil microorganisms; however, the majority of soil microbes are recalcitrant to cultivation but may be accessed using a metagenomic approach.

Objectives

We aimed to: 1) construct large-insert soil metagenomic libraries in shuttle BAC vectors that enable expression in multiple heterologous hosts, 2) screen libraries for antibacterial activity when expressed in *Escherichia coli* or *Sinorhizobium meliloti*, and 3) identify cloned genes encoding for 16S rRNA or Type I Polyketide Synthases (PKSs).

Materials and Methods

We used a random shear method to generate large insert metagenomic libraries in shuttle BAC vectors. Libraries were screened for antibacterial activity using an *in situ* bioassay or by soft agar overlays. Sequence-based screening used PCR or hybridization of library macroarrays, and specific clones were sequenced by bar-coded Next-Gen sequencing.

Results

We generated metagenomic libraries containing > 120,000 clones with average insert sizes >100 kb in shuttle BAC vectors. Sequence-based screening identified diverse 16S rRNA gene sequences affiliated with nine bacterial phyla, and PKS pathways with a broad range of homology (35-85%) with known PKS domains. A screen of 19,200 *E. coli* clones for inhibition of growth of methicillin-resistant *Staphyloccocus aureus* (MRSA) using an *in situ* lysis method yielded a total of 32 anti-MRSA clones. Sequence analysis revealed genes predicted to be involved in various biosynthetic pathways as well as many with no significant GenBank similarity. Interestingly, multiple clones were capable of modifying the chloramphenicol added to the culture medium, thereby resulting in modification of an existing antimicrobial scaffold. The metagenomic library was also conjugally transferred into *S. meliloti* and screened for activity against multiple bacterial pathogens. The genetic and biochemical characterization of antimicrobial expressing *S. meliloti* clones is ongoing.

Conclusion

These results illustrate that large-insert soil metagenomic libraries can be screened using sequence-based and innovative functional screening methods to access previously undescribed genomic and biochemical diversity.

OR1

NoDe and CATCh: two algorithms to get more accurate 16S rRNA sequencing data.

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The revolution in new sequencing technologies has led to an explosion of possible applications, including microbial biodiversity studies in the environment by bacterial 16S rDNA sequencing. However all sequencing technologies suffers from the presence of erroneous sequences, i.e. (i) chimera, introduced by wrong target amplification in PCR, and (ii) sequencing errors originating from different factors during the sequencing process. As such, there is a need for effective algorithms to remove those erroneous sequences to be able to accurately assess the microbial diversity.

First, a new algorithm called CATCh (Combining Algorithms to Track Chimeras) was developed integrating the output of existing chimera detection tools into a new more powerful method. Second, NoDe (NoiseDetector) was introduced as an algorithm to correct existing sequencing errors, thereby decreasing the number of reads or nucleotides that is disregarded by the current state-of-the-art denoising algorithms. Third, NoDe and CATCh were combined with a straight-forward pre-processing approach, creating a 454 16S rRNA sequencing analysis pipeline (software freely available and easily ingrated in pipelines like Mothur).

Via a comparative study with other chimera detection tools, CATCh was shown to outperform all other tools, thereby increasing the sensitivity with up to 14%. Similarly, NoDe was benchmarked against state-of-the-art denoising algorithms, thereby showing significant improvement in reduction of the error rate (reduction of 15 to 55%), combined with an minimal rejection of sequencing data retained after cleaning (20% more) and decrease in computational costs (90% faster). The cut-off parameters needed for the analysis of 454 pyrosequencing data were optimized, based on a Mock community (i.e. a known mixture of 18 bacterial species). Subsequently, this pipeline was used to study from the microbial diversity in borehole water of two different subsurface clay layers i.e. Boom clay (Mol, Belgium) and Opalinus clay (Mont-Terri, Suisse).

Conclusively, Introducing CATCh and NoDe into an existing 454 pre-processing pripeline, increaseses the overall reliability of the data. Further work implies fine-tuning toward other platforms (e.g. Illumina MiSeq).

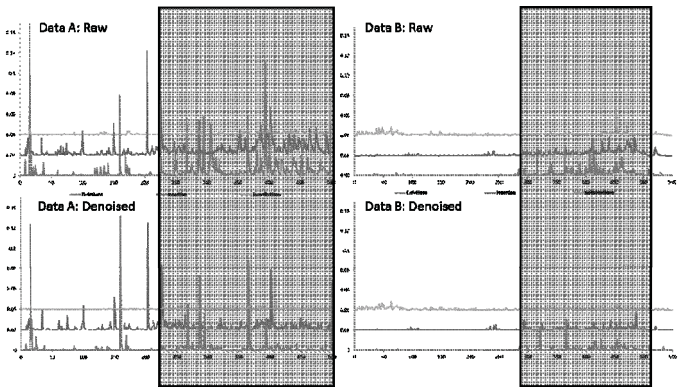

Figure 1. Plot showing different error types (substitutions, deletions, mismatches) along the position in the read, before and after NoDe.

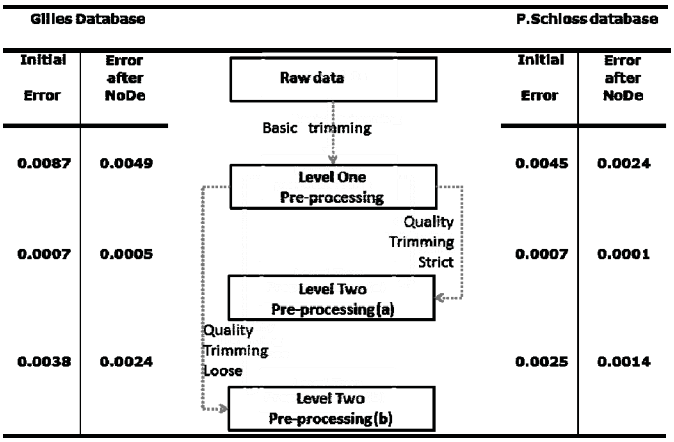

Figure 2. Flow chart showing three different pre-processing steps implemented in NoDe algorithm that aim at reducing the error rate of Gilles and P.Schloss datasets

OR2

Expanding the bioinformatic toolbox for the analysis of complex metagenomes

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Introduction

Metagenomics has revolutionized microbiological studies during the past decade and provided new insights into the diversity and metabolic potential of natural microbial communities. However, the tools to analyze metagenomic data are clearly lagging behind the developments in sequencing technologies (and data) and are typically limited to the assembly and gene annotation of the metagenomic sequences.

Objectives

Our overarching objective is to develop quantitative whole-genome approaches that fulfill critical needs of contemporary metagenomic research and scale with the high volumes of data that become available.

Materials and Methods
We developed new conceptual approaches for the analysis of metagenomes and the accompanying bioinformatic tools and applied them to *in-silico* generated (mock) datasets as well as publicly available metagenomes, including soil ones.

Results
Our bioinformatic tools can accomplish the following tasks: i) determine the level of sequence coverage obtained and the amount of sequencing required to obtain complete coverage (Nonpareil tool); ii) identify the taxonomic affiliation of a metagenomic read or assembled contig with unprecedented accuracy (MeTaxa); and, iii) identify (bin) assembled contigs that belong to the same natural population in time series metagenomic data (BinGeR). Most of our tools do not depend on the type of sequences available or can be easily adjusted to fit different types of sequences, and are freely available; for instance, through our lab website:http://www.enve-omics.gatech.edu/). Application of the tools to real metagenomes allow us, for example, to rank microbial communities in terms of their complexity and identify novel species that are abundant in-situ but have no sequenced representatives.

Conclusion
The work presented here provides practical means to perform various metagenomic analyses of microbial communities characterized by varied levels of diversity and efficiently handle the resulting data.

OR3
Assembly of a soil derived microbial consortium using a massive shared-memory computational system reveals novel bio-industrial enzymes

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Currently the soil microbiome is estimated to contain at least 1 terabase of unique genomic content, making it one of the most diverse microbioal ecosystems on earth. Understanding this ecosystem is critical to addressing the looming challenges of the 21st century including climate change, suitable agriculture, bioremediation and environmental friendly fuel production. Metagenomic sequencing, assembly and analysis of such a diverse community currently presents a major obstacle preventing further understanding and analysis of the soil microbiome. Here we show that the unique cache coherent Non-Uniform Memory Architecture (cc-NUMA) of the XSEDE resource "Blacklight", housed at the Pittsburgh Supercomputing Center (PSC), allows efficient genomic analysis of very large soil metegenomic datasets outside the scope of other high performance computing systems. Using a bioreactor seeded with a Brazilian sugar-cane field soil microbome, a lignocellulosic enrichment was conducted for 8 weeks on minimal media supplemented with a complex lignocellulotic plant material, sugar-cane bagasse, as the sole carbon source. 300 gigabase (GB) of sequence data was generated from the final community and assembled using Velvet on the XSEDE SGI Altix UV resource, Blacklight. This assembly produced 1.2 GB of assembled contigs greater than 300bp having a 2.3KB n50, a largest contig of 230kb, and using 3.6TB (3,600GB) of RAM. Phylogentic analysis of the produced contigs revealed a highly diverse community composition with over 70 phylum level groups present. The top four most abundant members reflected the dominant phylum members of soil found in previous 16s studies (Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutres) demonstrating the ability for this method to achieve sequencing of a soil community. Gene calling using the program MetaGeneMark produced 2.2 million peptide gene models which were functionally annotated using a combination of NCBI Blast and HMMMER/PFAM 26.0. Domain analysis using a conservative PFAM e value of e-4 yielded a large number of bio industrially relevant enzymes from the community including glycosyl hydrolases (32,143), Carboxylestrases (14,407), and Pectin Lyase (6,662). Further refinement of assembly algorithms and hardware optimization will allow assembly of 1terabase or more raw sequence data and move towards the holistic assembly of the soil microbiome.

OR4
Taxonomical and functional microbial community selection in soybean rhizosphere

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Niche theory and neutral theory have been raised to explain the microbial community assembly in a variety of environments. However, no theories have been applied for rhizosphere microbial community assembly. Using shotgun metagenomics approach we investigated the taxonomic and functional diversities of microbial communities in rhizosphere and bulk soil associated to soybean. Our results showed a power of taxonomic and functional selection over the soybean rhizospheric community assembly. The taxonomic analysis revealed that rhizosphere is a subset of the bulk soil community, with similar community composition but different structure. Rhizosphere samples showed an enrichment of bacterial phyla *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Cyanobacteria*, *Chlamydiae*, *Tenericutes, Deferribacteres, Chlorobi, Verrucomicrobia* and *Aquificae*. Species abundance in rhizosphere fits the log-normal distribution model, which is indicator of niche-based process. In addition, the data indicate that the rhizosphere community is selected based on functional cores related to metabolisms of nitrogen, iron, phosphorus and potassium, which may be related to benefits to the plant, as growth promotion and nutrition. Still, the network analysis involving bacterial groups and functions was less complex in rhizosphere, suggesting the specialization of some specific metabolic pathways. We conclude that the microbial assembly in the rhizosphere is based on niche process through a selection in the community composition in the rhizosphere.

OR5
Spatial structure of microbial communities across scales

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The spatial variability of soil microbial communities is critical for emergent properties that affect ecosystem function across scales. Furthermore, because of the relatively slow rate of mixing in soils,

an understanding of spatial heterogeneity is needed before assessments of temporal heterogeneity can be confidently separated from potentially confounding spatial variation. However, the spatial heterogeneity of microbial community structure and diversity is poorly understood owing to the destructive and labor-intensive nature of traditional soil sampling and processing methods. To better understand the spatial variation of soil microbial communities, we collected "microsamples" of soil (<3 g each) that dramatically enhanced the spatial resolution of diversity metrics. We collected twenty-five soil samples in a 10x10 cm grid at five points along a 36 m transect in each of six 36 x 20 m plots of a low-diversity perennial grassland dominated by *Panicum virgatum* (fertilized and unfertilized treatments) for a total of 750 soil samples. We sequenced the 515-806 region of the16S rRNA-encoding gene from each of the samples using the Illumia MiSeq to obtain information on soil bacterial and archaeal community structure. 5.23 million reads passed the initial quality filter and were used for subsequent microbial ecology analyses. We found that the diversity (Shannon index) of soil bacterial communities increased significantly with mineral fertilizer, driven by a significant decline in the relative abundance of Verrucomicrobia, the dominant phylum. Considerable patchiness in the abundances of the most dominant taxa emerged at the centimeter scale, but this variability was not due to spatial autocorrelation. We will also present an analysis of the contribution of this single dataset to the soil diversity represented in the whole Earth Microbiome Project database. This work will increase our understanding of spatial variations in microbial community structure and will link this structure with variations in concurrently measured abiotic factors. Overall, this effort is a first step in building greater mechanistic understanding of the feedbacks between soil microbes and ecosystem processes, such as carbon fluxes and nutrient cycling.

OR6
Micro-scale patterns of bacterial diversity

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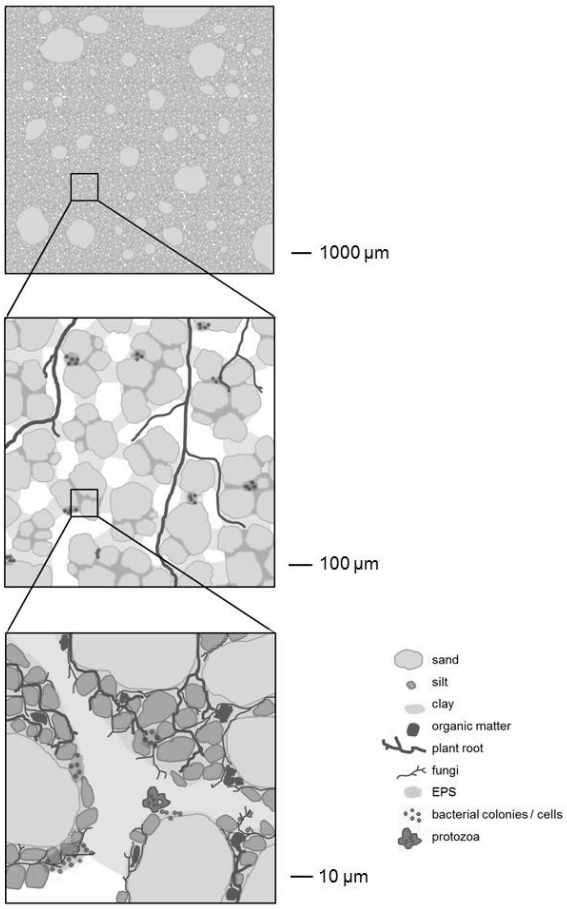
Soil-borne microbial diversity is vast. Although the influx of molecular, and more recently high-throughput sequencing, approaches has greatly expanded our appreciation of soil-borne microbial diversity, we still have a rather poor understanding the forces that drive patterns of microbial diversity and community structure in soil environments. To a large extent, this knowledge gap may be attributed the general failure to appreciate the scale at which the majority of microbial interactions occur, with most studies confined to spatial scales that are orders of magnitude larger than those most relevant to the microbes themselves. We have tried to address this issue via the combined application of survey-based and experimental approaches. In the former approach, spatially explicit sampling schemes down to the level of individual microaggregates have been combined with community analysis and pyrosequencing methods to determine the distribution of microbial taxa and functions at the micro-scale. In addition, a series or laboratory experiments have been conducted to determine the impact of soil structure on patterns of bacterial interactions and the maintenance of biodiversity. These experiments utilize well characterized artificial soil systems differing in grain size, soil moisture and connectivity that are inoculated with either defined mixtures of bacterial strains or complex communities of bacteria and bacteria and fungi. Population dynamics and community structure are then monitored over time, via selective plating and HTP sequencing approaches to determine the impact of specific soil conditions on microbial interactions and diversity. Results highlighted the importance of micro-scale heterogeneity in soil microbial community assembly and the impacts of soil moisture and connectivity on the distribution of microbial taxa with different life-history strategies.



OR7
Spatial analysis of bacterial communities in soil: the effect of spatial distance and environmental perturbation.

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Soils are among the most microbial diverse ecosystems on Earth due in part to the heterogeneity of their physical-chemical characteristics (*e.g.*, pH, humidity, temperature, organic carbon, etc.) Yet, different soils have had some of their DNA sequenced and a range of specific soil functions (e.g., nitrogen fixation, aerobic respiration, etc) measured. Correlations between physical-chemical characteristics and microbiological measurements have established some trends, although these might be a spatial scales far greater than their real variability. Some studies have linked the spatial diversity of some soil microorganisms with specific physicochemical parameters (*e.g.*, relationship between soil pH and *Acidobacter* abundance). If the physical-chemical characteristics are driving microbial community structure, then the heterogeneity of the bacterial community composition should vary at the same spatial-scale as the soil chemical property

heterogeneity. We focused our study on the small scale (sub-gram size). We studied the spatial distribution of bacteria in a soil core (30cm diameter) in order to understand the relationship between genetic diversity of microbial communities, spatial distance and soil physicochemical parameters. Half of the core was contaminated by hydrocarbons prior to distance-based sub-sampling. The microbial communities in the 60 subsamples were analyzed by 16S rRNA gene pyrosequencing. Both bacterial phylogenetic diversity and community function were evaluated. A 3D model of the core based on a Geographic Information System (GIS) approach was created to perform spatial analysis. The results of 16S rRNA gene analysis provided evidence of the relative importance of spatial distance and hydrocarbon pollution on the distribution of soils microbial communities. Initial spatial 3D modeling of the soil chemical characteristics and microbial community distribution using a geographic information system (GIS) and geostatistical tools quantified the relative importance of the different parameters.

OR8
Revealing microbial climate control: ecophysiology of sulphate-reducing microorganisms in peatlands

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Peatlands are a major source of the greenhouse gas methane and at the same time an important organic carbon reservoir. Their positive and negative feedback cycles to global warming and increasing aerial sulphur pollution are one of the largest unknowns in the upcoming decades to centuries. Although regarded primarily as methanogenic environments, biogeochemical studies revealed a hidden sulphur cycle in peatlands. Dissimilatory sulphate reduction is thermodynamically favourable relative to methanogenic processes and often occurs at rates comparable to marine surface sediments. Thus it effectively controls gross production of the greenhouse gas methane in peatlands. Our previous work revealed that rare *Desulfosporosinus* species have the potential to substantially contribute to sulphate reduction in an acidic peatland. To further our understanding of sulphate reducer ecophysiology in peatlands, anoxic microcosms were supplemented with typical degradation intermediates of organic matter at in situ concentrations and incubated under methanogenic or sulphate-reducing conditions. Microbial community dynamics and activity were analysed by highly parallel 16S rRNA gene and cDNA amplicon sequencing, quantitative real-time PCR, and metatranscriptomics. In addition, sequencing of a metagenome enriched by DNA-stable isotope probing allows functional analysis of the rare peatland *Desulfosporosinus* species. Amendment with different substrates resulted in varying turnover rates of sulphate, where the strongest effect was achieved with butyrate, followed by propionate, lactate, formate, and the weakest with acetate. Several microbial taxa/phylotypes responded positively to sulphate reduction. For example, *Desulfosporosinus* 16S rRNA copy numbers strongly correlated with sulphate turnover under all tested substrates, while we observed a significant increase of 16S rRNA copy numbers of *Desulfobulbaceae* only in response to butyrate and sulphate amendment. Dominating microbial populations such as *Acidobacteria* showed no significant response to sulphate turnover. As the abundance of all identified sulphate reducers was well below 1 % in the natural peat soil, our results provide further evidence for the importance of the rare bacterial biosphere to biogeochemical cycling and climate change.

OR9
Microbial diversity of dryland soils and its role in greenhouse gas turnover

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Drylands are the largest terrestrial biome on Earth, covering over 30% of the land surface and rapidly expanding through desertification. Low precipitation rates limit plant cover and permit the development of a unique microbial mat termed biological soil crust (biocrust), which is responsible for most of the photosynthesis and nitrogen fixation in these ecosystems. Biocrusts are dry and inactive throughout most of the year but short and sporadic pulses of rain lead to a burst of microbial activity followed by a return to dormancy upon dry out. A large body of literature exists on the photosynthetic and fungal members of these lifeforms, yet all other bacterial and archaeal inhabitants were only sporadically mentioned and have rarely been the focus of research. We previously showed that desert soils harbour distinct microbial communities which are different from more humid soils of similar geological origin. Through a combination of gas measurements and carbon isotope analysis with molecular approaches such as environmental gene expression analysis and ^{18}O-based RNA stable isotope probing coupled with 454 sequencing we show that biocrusts harbour two functionally distinct microbial communities, which resuscitate in a successional manner following rehydration. The top half of the biocrust remains oxic when wet and the succession following hydration is characterised by a rapid decline of the dominant Actinomycetales, which are then replaced by Sphingobacteriales and several Alphaproteobacteria (Rhizobiales, Rhodobacterales, Rhodospirillales and Rubrobacteriales). Cyanobacteria, the conspicuous members of biocrusts on the other hand, only constitute a fraction of these active microbes. The succession in the anaerobic part of the crust begins with activation of Bacillales, which are later replaced by Clostridiales. Methanogens of the genera *Methanosarcina* and *Methanocella* resuscitate last and produce CH_4 from hydrogen and CO_2. Since no methanotrophs could be found in biocrusts, the methane formed is likely to be released to the atmosphere. Our studies show that deserts host a diverse microbial community whose members display complex interactions and perform various biogeochemical processes as they resuscitate from dormancy. Furthermore we demonstrate the importance of understanding the links between functional responses of desert microbes and community formation in the face of environmental change. This will be of crucial importance if we aim to mitigate and predict the consequences of desertification.

OR10

Integrating "omics" to understand soil C cycling responses to precipitation variation

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Grassland soils (mollisols) store more than 30% of the terrestrial organic C in the continental U.S. and are expected to experience dramatic changes in precipitation patterns with climate change. Our project used three complementary omics approaches to evaluate the impact of altered rainfall patterns on microbial C cycling in a Kansas native prairie soil.

The study used the Rainfall Manipulation Plots (RaMPs) experiment at the Konza LTER, which was established in 1997. It maintains ambient and altered precipitation timing on intact prairie using an array of rainout shelters and sprinklers. Through every growing season, altered treatment soils receive an equal amount of water, in larger and less frequent events, than ambient soils, thereby extending the drought periods experienced by soil microbial communities. Soils were sampled during a wet-up event (before, 1 and 5 days after water addition) two times in 2011 (early and late summer). We measured the functional response of the microbial community along with the omics response (metatranscriptome and metaproteome). The omics data were analyzed using a custom, "active metagenome" based on sequencing BrdU-labeled DNA in conjunction with a comprehensive manually curated and validated database focused on specific biochemical functions and pathways of importance for soil microbial ecology.

The use of BrdU-labeled DNA improved assembly and enhanced the identification of transcripts and proteins from metatranscriptome and metaproteome datasets compared to a metagenome built from unlabeled DNA. Microbial functions responded to wet-up events differently in the early and late summer. For example, microbial C-use efficiency was higher in altered than ambient and also higher in dry (September) soils, and C-acquiring enzyme activities (β-glucosidase, cellobiohydrolase, and phenol oxidase) increased after rainfall in moist (June), but not dry (September) soils. These differences were often mirrored by metatranscriptomic responses. For example, the relative abundances of transcripts associated with oxidative phosphorylation showed a similar response to wet-up as did soil microbial respiration and transcripts for cellobiose transporters showed similar trends as β-glucosidase activity

OR11

Long-term effects of N, P, and K fertilization on the composition and function of bacterial communities in grassland soil

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What are the links between soil physicochemical parameters and the taxonomic composition and functional diversity of soil bacterial communities from long-term N, P, or K fertilization treatments?

We quantified 29 physicochemical soil parameters in 54-year fertilized grassland plots from five fertilizer treatments: N-, P-, K- and NPK-fertilized and non-fertilized control. We combined 16S rRNA and shotgun metagenomics to compare taxonomic structure and functional diversity of three replicate bacterial communities from the five fertilizer treatment groups. The MG-RAST server was used to generate taxonomic and functional profiles of BLASTx sequence comparisons to RefSeq and SEED databases for the taxonomic and functional analyses, respectively. Multivariate statistical analyses were conducted to investigate the links between soil parameters, microbial community structures and functions.

Community structures shifted between different fertilizer treatments while functional profiles were similar. At the taxonomic level of phyla, *Actinobacteria* were overrepresented in the bacterial communities from the N-, K, and NPK-fertilized treatments compared to the control treatment (p<0.05). The N-amended treatment communities exhibited the most difference from the control.

Of the treatments, the N-fertilized treatment showed the largest shift in community structure from the non-fertilized treatments, which supports the copiotroph-oligotroph theory of N-amendment. Overall, our results demonstrate functional redundancy of soil bacterial communities under long-term fertilizer amendment in grasslands.

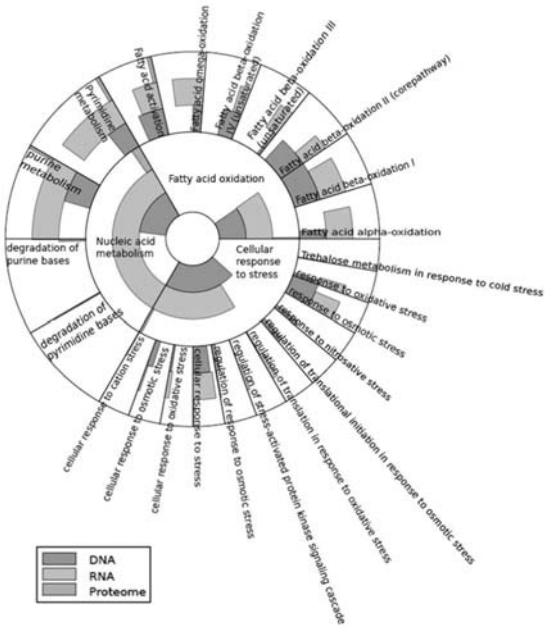

Figure1. Spearman correlation network between environmental parameters and community composition based on 16s rRNA fragment sequences. Significant correlations are presented (r>|0.6|, p<0.05).

OR12
The response of soil microbial diversity to long-term organic and conventional farming

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Sustainable farming practices are essential to maintain ecosystem functionality, preserve biodiversity, and meet economic demands. In this light, the Swiss DOK long-term agricultural management experiment has been initiated in 1978 in order to compare biodynamic, bio-organic, and conventional farming practices. Initial research focused on feasibility and productivity of organic farming, but more recent emphasis has been given to farming-related effects on soil quality. Since microbial processes regulate soil ecology and biogeochemistry, microbial diversity might serve as indicator of sustainable management. We have previously reported on findings based on genetic profiling (T-RFLP and RISA) of microbial ribosomal loci. These data have revealed consistent effects of long-term farmyard manure application and short-term crop cultivation; however, effects were more consistent for bacteria than for fungi. A classical Sanger sequencing approach yielded indications for specific bacterial groups that responded to the management influences, but insufficient diversity coverage and sample throughput did not allow for determining robust management indicators. Recent developments in sequencing technologies stimulated a reassessment of this site at much higher resolution and we re-analyzed the same soil samples using a massively parallel pyrosequencing approach targeting bacterial (16S) and fungal (ITS) ribosomal markers. We recovered a total of 1,118,268 bacterial and fungal pyrotags from 80 soil samples, revealing predominant abundances of Actinobacteria (30%), Proteobacteria (29%), and Acidobacteria (10%) as well as Ascomycota (53%), Basidiomycota (17%), and members of the former Zygomycota (17%). Massive sequencing supported the results from the previous studies, but revealed a substantial increase in resolution by identifying effects that went undetected using the traditional profiling approaches. Fertilizer application, crop protection regime, and crop cultivation were identified as major factors driving the structure of the soil microbiome. Network association analysis identified a range of microbial taxa that were significantly affected by the management practices, providing a first basis to elucidate long-term farming effects on the soil microbiome.

OR13
Organic carbon mineralisation in arctic peatsoils: key functions and microorganisms

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Question
A substantial part of the Earths´ soil organic carbon (SOC) is stored in Arctic permafrost-affected peatlands, which represent large potential sources for increased emissions of the greenhouse gases CH_4 and CO_2 in a warming climate. The microbial communities responsible for the mineralization of SOC to CO_2 and CH_4 in these soils and their response to a warming climate are, however, poorly understood.

Methods
We aimed to shed light on the key microorganisms and metabolic pathways active in SOC mineralisation of two Arctic peatlands from Ny-Ålesund, Svalbard using integrated "meta-omics" approaches. The influence of temperature on anaerobic SOC decomposition was investigated in microcosms experiments.

Results
The gene pool for plant polymer degrading enzymes was not different to the ones found in metagenomes of soils from other climatic zones. The majority of these genes were assigned to three bacterial phyla, Actinobacteria, Verrucomicrobia and Bacteroidetes. Genes and transcripts of anaerobic metabolic pathways and the fraction of methanogenic archaea increased with depth, suggesting a gradual transition to anaerobic lifestyles. Methanotrophic bacteria closely related to *Methylobacter tundripaludum* comprised more than 2% of the microbiota. The influence of temperature on anaerobic SOC decomposition was investigated in microcosms experiments. CH_4 accumulation and hydrolytic enzyme assays showed a non-linear response to temperature with high activities already at low temperatures. This was reflected in shifts of the microbiota structure and function at several key-positions in the anaerobic degradation pathways leading to CH_4 and CO_2.

Conclusions
This study contributes to a better understanding of microbial key populations in Arctic peatsoils and on their possible response to increased temperatures.

OR14
Comparative metagenomics of disease suppressive soils

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Natural control of soil-borne plant pathogens in disease suppressive soils has been documented for decades in various agricultural systems. Contrary to what is observed in disease conducive soils, suppression is characterized by a low disease incidence in spite of the presence of a susceptible plant host and a virulent pathogen. This phenomenon has been shown in many cases to be biologically-based, however our understanding of the microorganisms, microbial interactions and mechanisms underlying disease suppressiveness of soils is limited. Analysis of 16S rDNA amplicon libraries from a soil suppressive to *Fusarium oxysporum* f. sp. *lini* on flax revealed structural shifts among rhizosphere bacterial communities in suppressive soil in the absence and in the presence of the pathogen, as well as for a disease conducive soil. Comparisons of suppressive and conducive soils identified 25 differentially represented bacterial families, out of the 166 that could be identified among all treatments. Ten families were found at higher relative abundance in the conducive soils (such as *Flavobacteriaceae*, *Phyllobacteraceae*, or *Hyphomicrobiaceae*) and 15 were present at higher abundance in the suppressive soil (e.g *Cytophagaceae, Comamonadaceae, Oxalobacteriaceae* or

Sphingobacteriaceae). Furthermore, the *Sphingobacteriaceae* family also showed a significant increase in the suppressive soil in the presence of the pathogen (vs the non-inoculated S soil). Interestingly, members of this group, such as some *Sphingobacterium* or *Pedobacter* isolates were previously described as antifungal agents. Corresponding metagenome libraries were generated from the Fusarium wilt suppressive soil and from soils suppressive to *Rhizoctonia solani* to i) gain further insight into the functional genes present in these communities, ii) identify the taxa and functional genes that correlate with the level of disease control in each system, and iii) determine if a core disease suppressive rhizosphere microbiome exists. Up-to-date results of the individual and cross-comparative analyses will be presented.

OR15
Habitat filtering and competitive exclusion of large clades underlie phylogenetic clustering in soil bacteria

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Introduction
Soil bacteria typically coexist with close relatives resulting in widespread phylogenetic clustering. This has been attributed to the abiotic filtering of organisms with shared ecological tolerances.

Objectives
We investigated whether competition can also explain the phylogenetic similarity of coexisting bacteria by excluding large clades with low competitive abilities (Mayfield & Levine 2010 Ecol Lett 13, 1085-93).

Materials and Methods
We used a trait-based approach to discern between abiotic and biotic factors underlying phylogenetic clustering in soil bacterial communities in a model system. We collected soils in semi-arid plant patches and adjacent bare areas, which shape a mosaic of habitats of highly contrasted productivity and comparatively dominated by biotic and abiotic processes. We pyrosequenced the bacterial 16S rRNA gene and examined the distribution of 3290 bacterial operational taxonomic units (OTUs) in "patches" and "gaps" (n = 15). For each OTU, we recorded the presence (or not) of ten traits associated to tolerance to abiotic stress (tolerance to desiccation, salinity, and formation of resistant structures) and competitive abilities (heterotrophy, phototrophy, N fixation, nitrification and/or denitrification, S oxidation, S reduction, and formation of stalks) based on a literature review. We analysed the environmental distribution of all traits and their phylogenetic conservatism.

Results
Bacterial traits associated to abiotic stress tolerance (desiccation and salinity) were overrepresented in low productive habitats, while competition related traits (carbon usage) prevailed under high resource availability. In this carbon-rich soils, Proteobacteria were overrepresented, while four other major phyla were underrepresented. This likely responds to Proteobacterial superior competitive ability under carbon enriched conditions (Goldfarb et al. 2011 Front Microbio 2, 1-10). All traits were phylogenetically conserved (as in Martiny et al. 2013 ISME J 7, 830-838). Phylogenetic clustering was stronger in habitats comparatively dominated by biotic processes.

Conclusion
Competitive exclusion of large bacterial clades, based on asymetric competition for carbon, might also underlie the ecological similarity of co-occuring soil bacteria.

OR16
The strength of phylogenetic signals in microbial traits: A proof of principle as applied to methane-oxidizing bacteria

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Understanding the relationship between species composition and ecosystem function is fundamental to predicting ecosystem responses to global change. In biodiversity ecosystem functioning research, there has been a transition from classical taxonomic approaches towards the application of functional traits or phylogenetic inference. Here we tested whether a straightforward relationship between phylogenetic identity and functional trait values exists for microorganisms, using aerobic methane oxidizing bacteria (MOB) as a model system. We generated a database which included 50 MOB strains with quantitative functional trait information. We used a method from comparative biology to calculate the phylogenetic signal based on the *16s rRNA* gene, a common phylogenetic marker, and the *pmoA* gene which encodes a subunit of the key enzyme involved in the first step of methane oxidation. The phylogenetic signal of functional traits of MOB was different from traits evolving under a Brownian motion model of evolution. Functional traits associated with metabolic activities had a more pronounced phylogenetic signal with the *pmoA* than with the *16s rRNA* phylogeny. In conclusion, our results suggest that current trait-based approaches entirely based on taxonomical information will not yield further advancement on the relationship between microbial species composition and ecosystem function. To generalize these results, the phylogenetic signal of quantitative traits should be tested in other microbial groups as well.

OR17
Reconstruction of full-length SSU rRNA genes from DNA/RNA-seq data profiles the three domains of life in soil environments

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Introduction
Here, we chose biological soil crusts (BSCs) and a grassland rhizosphere as model soil systems. Our goal was to demonstrate the use of non-primer/PCR biased SSU rRNA gene reconstruction to integrate the study of all domains of life in soil from DNA- and RNA-seq data.

Material and Methods
BSCs were collected from the Moab desert (Utah, USA). These were subjected to a wet-up event prolonged for a day/night cycle. The metagenome was constructed with DNA collected prior wet-up. The metatranscriptomes were constructed with RNA collected 18h (nighttime) and 25.5h (daytime) after wet-up. *Avena fatua* plants were grown in a microcosm experiment and rhizosphere and bulk soil RNA were collected 8 weeks after the experiment started. We used the EMIRGE package to reconstruct SSU rRNA genes from DNA/RNA seq data.

Results
In the BSC experiments, we were able to reconstruct over 244 SSU rRNA OTUs from the metagenome, and over 4,700 OTUs (175 Eukaryotic) from the metatranscriptomes. BSCs were dominated by Cyanobacteria, especially *Microcoleus vaginatus*. Moreover, because we were able to reconstruct OTUs belonging to Eukaryotes, we were able to detect Fungi, Protozoa, Plantae and Nematoda in our nighttime metatranscriptome.

The *Avena fatua* rhizosphere showed a much richer community when compared to BSCs. An average of 550 Bacteria and 370 Eurakyotes were observed in the metagenomes. Moreover, an average of 5,500 OTUs was observed for each rhizosphere and bulk soil sample. Fungi (Ascomycota and Mucoromycotina) were enriched in the rhizosphere and different Rhizaria were enriched in the bulk soil.

Conclusions
Our results suggest that a better understanding of soil microbial dynamics may be achieved when simultaneously studying the diversity of the three domains of life. These observations would not be possible without a non-PCR/primer biased parallel reconstruction of the SSU rRNA genes.

OR18
Functional soil metagenomics: elucidation of polycyclic aromatic hydrocarbon degradation potential after 10 years of *in situ* bioremediation

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Polycyclic aromatic hydrocarbons (PAHs) are among the most ubiquitous contaminants and pose a significant risk to humans and the environment. Microbial degradation represents the major mechanism responsible for the ecological recovery of PAH-contaminated sites.

Here we present a culture-independent approach to assess the microbial aerobic catabolome for PAH-degradation.

To study the microbial community of a PAH-contaminated soil subjected to 10 years of *in situ* bioremediation, the community structure was assessed by Illumina-based deep sequencing of amplicons targeting the V5-V6 region of the 16S rRNA gene. In a complementary approach, a metagenomic library was prepared in pCCFos and a total of 425 000 clones subjected to activity-based screening for key catabolic ring-cleavage activities using 2,3-dihydroxybiphenyl as substrate. Since most of the genes encoding extradiol ring-cleavage enzymes on 672 fosmids could not be identified using primers based on currently available sequence information, 200 fosmid inserts were sequenced using the Illumina technology.

To accurately annotate catabolic genes encoded by the environmental metagenome, we developed manually curated databases for catabolic key gene families involved in PAH-degradation. Using this bioinformatic tool, we successfully overcame the overwhelming level of misannotations in databases. Sequence information of the fosmid inserts revealed not only the presence of novel extradiol dioxygenase genes but also additional key genes of aromatic metabolic pathways only distantly related to previously described variants.

The given detailed framework of the metagenome dedicated to PAH-metabolism will serve as base for a careful analysis of the metabolic net acting on PAHs under real environmental conditions through metatranscriptomic analysis.

OR19
Cultivation and metaomics approaches characterize organohalide-respiring communities

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Organohalide-respiring bacteria use specific chlorinated hydrocarbons as electron acceptors, and their activity can lead to detoxification of priority pollutants. Organohalide-respiring *Dehalococcoides* and *Dehalobacter* strains rely on other community members to provide essential nutrients including hydrogen and corrinoid.

Question
What community members support organohalide-respiring *Dehalococcoides* and *Dehalobacter* and can specific functions be assigned to “helper” populations?

Methods
Enrichment cultures were obtained from contaminated site materials with different chlorinated solvents provided as electron acceptors and lactate as electron donor. Meta-genomic, -transcriptomic and -proteomic workflows combined with cultivation, 16S rRNA gene-based analysis and substrate utilization measurements characterized the source materials and enrichment cultures.

Results
Enrichment with chlorinated ethenes and chlorinated propanes consistently yielded cultures dominated by *Dehalococcoides*, whereas *Dehalobacter* strains were the predominant dechlorinators in cultures amended with chlorinated methanes or chlorinated ethanes. The known *Dehalococcoides* and *Dehalobacter* isolates are corrinoid auxotrophs even though a vitamin B12 derivative is essential for catalyzing reductive dechlorination reactions.

The analysis of defined co-cultures and enrichment cultures without exogenous vitamin B12 characterized *Dehalococcoides*' corrinoid requirements and unraveled community members supplying this critical cofactor. *Sphaerochaeta* spp. with strictly fermentative metabolism cannot synthesize corrinoid but were commonly observed in *Dehalococcoides*-containing enrichment cultures. The metatranscriptomic analysis revealed possible roles of these non-dechlorinating spirochetes for supporting the reductive dechlorination process, including protection from oxidative stress.

Conclusions
Repeated transfers with the same chlorinated electron acceptor yielded enrichment cultures dominated by strains of a specific organohalide-respiring genus. The diversity and structure of the non-dechlorinating community were comparable between enrichments. Specific function can be assigned to non-dechlorinating community members indicating that a reductively dechlorinating core community can be defined.

OR20
The evolutionary space of the bacterial 16S rRNA gene as a new operational field for integral metagenomics

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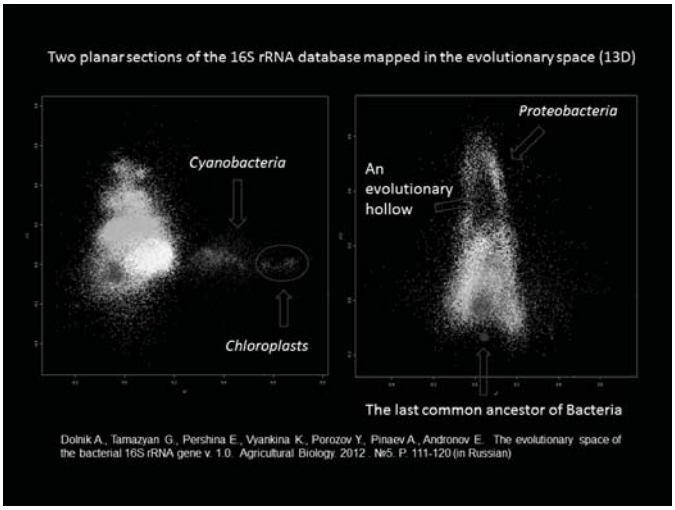
One of the oldest problem of biological systematics known as the natural system of organisms (Carl Linnaeus, 1783) despite the deep interest of many prominent biologists is still far from resolution. Such a system unlike a catalogue comprises every possible organism regardless of whether this organism is present in biosphere. Need for similar system created for a particular gene only is especially obvious in 16S rRNA ecology, where the proportion of not assigned sequences may be extremely high. OTU concept can only partly resolve this problem since it requires constant re-alignment of 16S rRNA sequences set when new sequences is added. On the other hand one of the most obvious requirements of metagenomics is the access to integral parameters describing complex communities as a whole.

In this report we are presenting a sketch of a new operational field for 16S rRNA ecology "the evolutionary space of 16S rRNA gene" which potentially can resolve both of these problems. In this space fixed coordinates exist for every possible variant of 16S rRNA. We proved that minimal dimension such a space is 13 (but the exact solution demands more dimensions). The algorithm proposed is based on search of regular simplexes in the distance matrix of 16S rRNA database. Each particular microbial community can be presented in this space as a cloud and a set of integral parameters can be calculated (volume, density, shape, the central point etc.). Integral parameters of succession may by presented as vectors and angles between vectors. We applied this technique to estimate dynamic of microbial communities in salinity gradient and to quantify the primer-bias effect.

But the most interesting results were evolutionary patterns identified when16S rRNA database had been mapped in the evolutionary space. For this purpose about 1000 thin planar sections were obtained. We probably identified "an evolutionary hollow" (the used area of the space where 16S rRNA ancestral sequences eliminated from biosphere in course of evolution were located).

One of the promising areas is construction analogous spaces for any protein coding genes. For this purpose creation of "supermatrix" (mix of DNAs collected form thousands environments) can be extremely useful.

The work was supported by RFBR 12-04-01371-a and SPbGU program for fundamental researches.



OR21
Amazonian resistome: evaluating antibiotic resistance abundance and diversity across a French Guiana forest soil gradient through metagenomic approaches.

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Antibiotic resistance gene determinants acquired by pathogenic bacteria are a serious healthcare issue. While this antibiotic resistance phenomenon is well studied in the clinical context, little information concerning the environmental resistome is currently available. However, a number of studies have reported the isolation of resistant bacteria from aquatic and soil samples. A better understanding of antibiotic resistance prevalence and diversity in the environment will help elucidate resistant gene movement between environmental and clinical pathogenic bacteria. Therefore, we focused on a well-preserved study site, the Trois-Sauts village located in Guiana Amazonian National Park to which access is restricted to permanent residents since 1970. Trois-Sauts is isolated from any sources of antibiotics except those provided by the village dispensary, which registers every prescription. A 3000m soil transect was sampled in triplicates every 600m resulting in 18 soil samples from the village to the forest. We hypothesized that a single antibiotic source could affect the soil bacterial community in terms of antibiotic resistance genes and mobile genetic element (MGEs) abundance. Soil metagenomic DNA was extracted from all samples and submitted directly to Roche 454 pyrosequencing, resulting in 18 datasets of metagenomic DNA sequences reads. Each dataset was annotated independently and antibiotic resistance genes were screened by

sequence homology to a reference database. We also performed quantitative PCR on the same DNA samples, focusing on some antibiotic resistance representative marker genes (such as blaSHV, blaTEM, sul(I), tet(G), tet(H), cfiA) already found in environmental samples. Mobile genetic element markers (such as int1, int2 for integrons and trA, korB and rep for conjugative plasmids) were also quantified. Correlations between the presence of mobile genetic elements and antibiotic resistance genes could be established. However, we did not observe a significant increase in antibiotic resistance gene load between distal (3000m) and village (0m) samples.

OR22
Unraveling the diversity and dynamics of the bacterial mobilome in on-farm biopurification systems

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Biopurification systems (BPS) used on farms are an efficient pollution control technique to treat water contaminated with different pesticides. It is assumed that mobile genetic elements (MGE) of the bacterial community present in a BPS contribute to adaptation of bacteria to changing environmental conditions, e.g. due to the addition of various pesticides. Therefore, the composition and shifts of the MGE carried by bacterial population in a BPS located in Belgium exposed to different concentration of pesticides over an agriculture season were investigated. In addition, a microcosm experiment was performed in order to evaluate the response of pre-adapted BPS bacterial communities to linuron. We hypothesized that the abundance of MGEs is positively correlated to the increase of pesticide concentration. Results of PCR amplification of MGE-specific sequences from total community DNA in combination with Southern blot hybridization and qPCR revealed a high abundance of several MGEs analyzed. qPCR showed an increased relative abundance of IncP-1 korB copies in response to linuron application but also in the BPS during the field season suggesting an increase in IncP-1 plasmids. Amplicon pyrosequencing of the IncP-1 trfA gene revealed a high IncP-1 plasmid diversity and suggested that populations carrying IncP-1β plasmids increased in abundance while those carrying IncP-1ε plasmids decreased in number in response to pesticide applications under microcosm and field conditions. IncP-1β plasmids were also exogenously isolated and sequence analysis is presently in progress. Pyrosequencing of 16S rRNA gene amplicons from TC-DNA obtained from samples taken in both experiments suggested the existence of a complex pesticide degrading bacterial food web in the BPS and that in particular IncP-1 plasmids might carry genes involved in the linuron degradation.

OR23
Production of secondary metabolites in soil and sand microcosms of *Streptomyces coelicolor*

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Streptomycetes are soil dwelling filamentous bacteria, known for their production of bioactive 'secondary' metabolites. Genome sequencing of the model species *Streptomyces coelicolor* identified 26 known and predicted (cryptic) secondary metabolite gene clusters, involved in the biosynthesis of multiple classes of bioactive compounds, e.g. antibiotics, siderophores, lipids, pigments and ribosomally-synthesized and post-translationally-modified peptides. Most studies investigating microbial secondary metabolite production are usually undertaken in liquid or on solid media. Information is lacking on gene expression and antibiotics production in soils. The aim of this study was to investigate primary and secondary metabolism in a model *Streptomyces* grown under natural conditions.

The results from proteomic, transcriptomic, and metabolomic studies of *S. coelicolor* grown in sand or soil microcosms with differing carbon, nitrogen and metal content will be discussed. Interestingly and contrary to the consensus of secondary metabolism commencing after reduction or cessation of growth, this study revealed expression of secondary metabolite biosynthetic genes and proteins at the onset of and during exponential growth in sand and soil. Some secondary metabolite genes/proteins were expressed constitutively which was further supported by metabolic data. The blue-pigmented antibiotic actinorhodin could be detected throughout the growth phases in sand cosms. 'Secondary' metabolism as a term should therefore be reconsidered under environmental conditions as this study suggests it plays an important primary role in nature.

OR24
Genomics and metagenomics for discovery of novel bacterial laccases

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Laccases oxidize a variety of phenolic compounds by reducing oxygen to water. They oxidize a broad range of substrates and are therefore highly interesting for environmental and industrial applications. These enzymes were mostly studied in fungi. In contrast, bacterial laccase-like enzymes are poorly understood and only few have been studied extensively. We have recently identified 1,200 putative laccase-like genes in 2,200 draft and completed genomes using custom profile Hidden Markov Models. Up to 76% of laccase genes identified encode signal peptides and

many are located on plasmids or associated with putative mobile genetic elements suggesting their mobility among bacteria. Moreover, the purification and characterization of two candidate laccases identified as interesting targets by bioinformatics approaches confirmed that novel genes encode laccases with different (high) pH optima and temperature resilience, thereby exemplifying the power of genomics in finding interesting biocatalysts. Recently, metagenomics was applied for the bio-mining of bacterial laccases in soil in collaboration with other partners within the METAEXPLORE project, and obtained results will be discussed. Emphasis will be given to exploration of the low pH bog soil metagenome leading to the discovery of the first acidobacterial laccase.

OR25

Next-generation biocontrol products: development and optimization by (meta)omics-technologies

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Rhizosphere and soil microbiomes can protect plants against both biotic and a-biotic stress. While biocontrol is mediated by bacteria and fungi that provide protection directly against plant pathogens or via the host plant themselves, stress protection by microorganisms is less understood. Although next-generation bio-products have an extremely fast-growing market potential world-wide; hurdles for commercialization are inconsistent effects, not understood mode of action and interaction and difficulties with fermentation/formulation but also with risk assessments. We try to solve these problems by applying (meta) genomics, metabolomics, and transcriptomics. Promising results were obtained for the stress protecting agent *Stenotrophomonas rhizophila* DSM14405^T. Using omics-technologies we found not only new substances involved in bacterial stress protection such as spermidine and glucosylglycerol, we could also identify a fine line between pathogens and beneficials within the genus *Stenotrophomonas*. We identified certain properties that indicate pathogens such as growth at human-relevant temperatures together with the production of heat shock proteins as opposed to beneficials that possess a temperature-regulated suicide system. In addition, rhizosphere microorganisms are great chemists and metagenomic approaches now allow exploiting the potential of so far uncovered metabolites for biocontrol products. Currently we are working on metagenomes of mosses and lichens to identify genes of bioactive compounds which can lead to the development and optimization of biocontrol strategies for different agricultural areas and applications.

P1

CopyRighter, improved quantification in microbial amplicon surveys through gene copy number correction

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Introduction

Culture-independent molecular surveys that target conserved marker genes like 16S ribosomal rRNA to assess microbial diversity are semi-quantitative due to variations in gene copy number (GCN) between species, from a single copy to as many as 15.

Objectives

In this work, we sought to develop a bioinformatic method to correct GCN in microbial datasets.

Materials and Methods

We calculated the GCN in all microbial genomes in the IMG database using Infernal and determined the phylogenetic signal of GCN in the Greengenes tree based on Pagel's λ. We used the phylogenetically independent contrast framework to compute GCN for the million of OTUs in Greengenes, and developed software, CopyRighter, that leverages these estimates to correct 16S rRNA amplicon datasets. We tested the accuracy of Copyrighter with *in silico* and *in vitro* mock datasets and demonstrated its usefulness by correcting previously published datasets.

Results

We found that GCN is strongly linked to microbial phylogeny, making it possible to estimate the GCN of microbial species based on that of their sequenced relatives. Our novel software tool, CopyRighter, uses pre-computed GCN estimates to quickly correct qPCR abundance and microbial profiles produced by common microarray or amplicon bioinformatic pipelines. Mock communities validated that the use of CopyRighter is beneficial to more accurately assess microbial relative abundance. Using Copyrighter, we were able to cluster human gut amplicons into three enterotypes instead of two,as supported by metagenomic data.We also unveiled that the biomass in an anaerobic digestor bioreactor remained fairly constant over a year, despite strong variations in uncorrected qPCR values.

Conclusion

CopyRighter effectively corrects GCN, making microbial surveys more quantitative. It alters estimates of total microbial abundance, α and β diversity, and is thus critical for drawing valid biological conclusions.

P2

StreamingTrim 1.0: a Java software for dynamic trimming of 16SrRNA sequence data from metagenetic studies

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Introduction

One of the most important problems related to the production and utilization of DNA sequence reads is the analysis of base quality and removal of low-quality segments, while retaining sufficient information for subsequent analyses. In metagenetic analyses, it is very important to preserve the integrity of each read as much as possible, while reducing the loss of important taxonomic information present in a 16S or 18S rRNA gene sequence.

Objectives

To overcome the limitations imposed by the existing trimming

software programs, we have developed StreamingTrim using standard Java language and BioJava. This software uses a very flexible approach which allows users to set a custom quality cutoff.

Materials and Methods
Simulations were performed on datasets of 16S rRNA amplicons comparing StreamingTrim with other trimming software. We introduced a trimming performance estimator (Z-score) proportional to the ratio between the increase in quality and the decrease in the number of bases. Taxonomic simulations were performed on a mock dataset, present in the NCBI SRA database, using RDP multiclassifier.

Results
In terms of computational time, StreamingTrim performed faster than most of the software programs. Obtained results showed that StreamingTrim had the highest Z-score values, indicating the presence of a good compromise between bases conservation and increase in reads quality. Moreover, taxonomic simulation showed that all tested trimming software produced very similar taxonomic patterns.

Conclusion
StreamingTrim has correctly assigned a very high proportion of reads to their taxonomic classification, while performing better than several other software programs. StreamingTrim provides several improvements over previously developed trimming software programs and could be considered a valuable alternative or a complement, especially when used by "wet lab" molecular ecologists.

P3
GnS-PIPE: an optimized bionformatic pipeline to efficiently assess microbial taxonomic diversity of complex environments using high throughput sequencing technologies.

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The rRNA genes (16S, 18S, ITS) are widely used to study microbial communities in soils, as they can be easily amplified from metagenomic DNA. Moreover, the recent development of high-throughput sequencing technologies allows the assessment of millions of sequences from a single metagenomic DNA.

Some pipelines are already available (e.g. QIIME or Mothur) to efficiently treat such data. However, the development of bioinformatic tools must now be validated by various biological tests. This was particularly true for key steps to appraise microbial diversity and richness. Here, we present a new pipeline named GnS-PIPE, a software application performing bacterial, archaeal and fungal taxonomic diversity analyses. One of the key design in the development of GnS-PIPE was that we conduct biological validations of defined bioinformatic steps. These biological tests have been performed using the expertise of the GenoSol platform, a biological resource centre unique in France, devoted to the conservation and analysis of the genetic resources of soil microbial communities.

GnS-PIPE includes several optimized steps, like a step to correct homopolymer errors due to the pyrosequencing technology, a biological approach to detect chimera sequences using a database of known sequences from various metagenomic studies, and a taxonomic assignation method merging results from various databases and methods. Moreover, a user-friendly graphical interface working on Windows, Mac OS and Linux systems was developed to easily interact with GnS-PIPE, and manage samples and analysis. To date, GnS-PIPE provides an optimized approach to easily analyse microbial biodiversity from widescale soil samplings.

P4
A workflow for analyzing taxon and pathway coverage profiles with applications to metatranscriptomics

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Introduction
High-throughput sequencing has recently resulted in an increasing number of studies focusing on complex meta*omic questions. Environmental factors influence on the one hand species richness and composition, on the other hand the functional repertoire of the whole ecosystem. To investigate both aspects, novel bioinformatic tools are needed to accurately and efficiently assign taxonomic as well as functional annotation to each sequenced short read.

Objectives
The objective of this study is to analyze metatranscriptomes under changing environmental conditions and investigate adaptation of populations and functions. As we do not want to focus solely on bacterial communities or certain markergenes, a new snakemake workflow was developed to annotate complete metatranscriptome datasets.

Materials and Methods
Snakemake is a workflow framework that allows to describe workflows via a readable and concise textual language with a syntax inspired by Python. Workflow steps are described in terms of rules that create output files from input files and execute shell commands, R or Python code. Snakemake automatically determines dependencies between rules and parallelizes independent tasks.

Results
The annotation process consists of two main parts, the assignment of reads (I) to taxa and (II) to genes and metabolic pathways. First, the reads are mapped with SHRiMP2 against a custom-built reference database to obtain taxonomic orders (e.g. phylum). Since public database are biased towards certain well-studied species, the reference database consists of sequences from an evenly spread set of species over all domains of life. Secondly, information on gene functions and metabolic pathways are retrieved by mapping the reads to the UniProt database using RAPSearch2. For the statistical analysis appropriate normalizations are applied to account for differences in reads per sample and sequence information per taxon. Comparative analyses between environmental conditions, e.g. salt concentration or heavy metal contamination, are then performed on basis of the sample-specific taxon coverage and pathway profiles.

Conclusion
We present a novel snakemake workflow as a tool to annotate complete metatranscriptomes and analyze them in terms of taxonomic and functional changes due to various environmental factors.

P5
Design and experimental validation of a novel non-degenerate universal primer set for 16S rRNA gene amplicon sequencing with a low possibility to amplify eukaryotic rRNA genes

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Introduction
The deep sequencing of 16S rRNA genes amplified by universal primers has revolutionized our understanding of microbial communities by allowing the characterization of the diversity of the uncultured majority. However, some universal primers also amplify eukaryotic rRNA genes, leading to a decrease in the efficiency of sequencing of prokaryotic 16S rRNA genes with possible mischaracterization of the diversity in the microbial community.

Objectives
We attempted to identify the universal primers that complement only conserved bacterial and archaeal 16S rRNA gene sequences so that eukaryotic and organelle rRNA gene sequences would not be amplified.

Materials and Methods
We compared 16S rRNA gene sequences from genome-sequenced strains and identified candidates for non-degenerate universal primers that could be used for the amplification of prokaryotic 16S rRNA genes. The 50 identified candidates were investigated to calculate their coverage for prokaryotic and eukaryotic rRNA genes, including those from uncultured taxa and eukaryotic organelles. A newly identified universal primer set was validated by the amplification of 16S rRNA genes from a soil metagenomic sample and subsequent pyrosequencing using the Roche 454 platform. The same sample was also used for pyrosequencing of the amplicons by employing a commonly used primer set, 338F-533R, and for shotgun metagenomic sequencing using the Illumina platform.

Results
Our comparison of the taxonomic compositions inferred by the three sequencing experiments indicated that our primer set can characterize the taxonomic composition of the microbial community without substantial bias, and consequently can be applied to a wide variety of microbial communities.

Conclusion
We identified a novel universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes.

P6
RDP- data and tools for soil microbial ecology

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RDP offers aligned and annotated rRNA sequence data and analysis services to the research community (http:// rdp.cme.msu.edu). These data and tools are widely used in fields beyond microbiology such as human health, agriculture, engineering, and environmental sciences. Updated regularly, RDP offered 2,765,278 aligned and annotated quality-controlled rRNA sequences and associated information as of May 2013. RDP provides a range of tools to help users analyze their own sequences using this rich knowledge base. These tools include the RDP Classifier and RDP Pipeline for rapid analysis of high-throughput amplicon data, including paired-end Illumina data.

While rRNA genes remain the most commonly used markers, key genes in ecologically important pathways can provide vital information of community composition and function not obtainable through rRNA analysis. However, working with such ecofunctional gene data requires tools beyond those required for rRNA analysis. The RDP Functional Gene Pipeline and Repository (FunGene; http://fungene.cme.msu.edu/) offers databases of many common ecofunctional genes and proteins, as well as integrated tools specialized for the processing of coding gene amplicon data, such as the FrameBot frameshift-correcting nearest-neighbor assignment tool. Together, RDP and RDP FunGene provide resources researchers require to query both phylogenetic marker genes and key genes involved in important soil processes.

In addition to web interfaces, many RDP and RDP FunGene tools are made available for download as Illumina-ready, stand-alone versions from https://github.com/rdpstaff. Tutorials are provided to guide researchers through the otherwise complex data processing steps in well-defined, task-oriented workflows with detailed instructions. RDP's mission includes user support; email rdpstaff@msu.edu or call +1(517)432-4997.

P7
Habitat specific adaptation of alphaproteobacteria based on GC content, genome size and aromatic dioxygenase gene distribution

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The α-proteobacteria class is the one of the largest sequenced group within phylum Proteobacteria (235 published genomes). They are versatile including numerous phototrophs, chemolithotrophs, chemoorganotrophs and aerobic

photoheterotrophs. The association of many α-proteobacteria with eukaryotic organism is of central importance in the agricultural and medical field. Symbiotic association of the Rhizobiaceae family bacteria with root nodules is responsible for most of the atmospheric nitrogen fixation by plants. Other α-proteobacteria such as Rickettsiales, *Brucella* and *Bartonella* have adopted intracellular life styles as human and animal pathogens.

In hydrocarbon polluted sites alphaproteobacteria are often dominating the microbial community and the unexplored genome capability of aromatic degradation it's of great interest. The aim was to study the flexibility in catabolic oxygenase genes and evaluate the relative importance of phylogeny versus habitat while considering the catabolic potential of alphaproteobacteria. Published genomes (235) of the Alphaproteobacteria class were selected for the study of the catabolic gene distribution in chromosomes and plasmids/chromids in relation to the habitat. Genome sequences and metadata were downloaded from various biological databases (NCBI, Patric etc) for the analysis.

The relative frequency of the aromatic degrading dioxygenases (DOs) is lower in alpha- than in betaproteobacteria (Figure 1), but the frequency of plasmid encoded DOs was relatively higher. The order Sphingomonadales had the highest frequency of plasmid encoded DOs (Figure 2). The frequency and occurrence of DOs showed that the microbes isolated from the rhizosphere and soil possess more catabolic genes compared to those isolated from other habitats. We found that species even of the same genus had varying genomic features eg; genus *Methylobacterium* isolated from air, soil, plants and rhizosphere. *Methylobacterium nodulans* ORS 2060 (rhizosphere) showed high GC content, larger genome size as well as higher frequency of catabolic genes compared to the other seven sequenced species. Habitat seemed to play a more important role in evolution of catabolic versatility in alphaproteobacteria than phylogenetic origin.

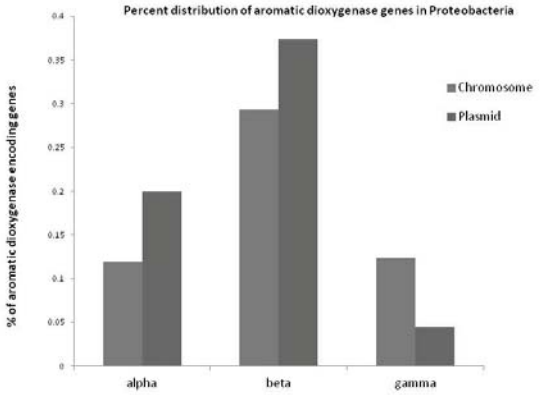

Figure 1. Per cent distribution of aromatic dioxygenase-encoding genes per total genes encoded in three different classes of Phylum Proteobacteria.

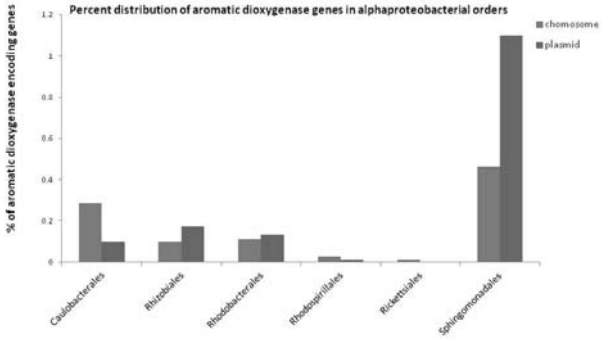

Figure 2. Per cent distribution of aromatic dioxygenase-encoding genes per total genes encoded in different Orders of class Alphaproteobacteria.

P8

Mixture models for the estimation of metagenomic abundances

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Metagenomics has become a standard approach to analyze microbial communities from environmental and clinical samples. The advances of next- generation sequencing technologies allow researchers to investigate the diversity even of complex microbial communities. However, this development demands new bioinformatics tools which can efficiently deal with metagenomic data sets on a large-scale.

We developed Taxy-Pro and a Mixture of Pathways (MoP) model for the estimation of taxonomic and metabolic relative abundances. Both methods provide a solid statistical basis and at the same time, a fast computation of taxonomic and metabolic profiles.

Taxy-Pro implements a mixture model based on protein domain frequenciesforinferring the taxonomic composition over the whole range of biological entities - including all domains of life and viruses. Our results indicate that Taxy-Pro estimates may provide important advantages when analyzing data with a high fraction of archaeal or viral DNA. Taxy-Pro is freely available at

http://www.gobics.de/TaxyPro/ as a Matlab/Octave toolbox or through the CoMet web server (http://comet2.gobics.de/).

The MoP model extends the taxonomic mixture model to a statistically adequate modeling of the metabolic potential of metagenomes. To overcome computationally intense homology searches, we implemented a shortcut to estimate the metabolic profile of a metagenome. Here, we link the taxonomic profile of the metagenome to a set of pre-computed metabolic reference profiles. The combination of the taxonomic abundance estimates and the metabolic reference profiles achieves an unrivaled speed of the metabolic profiling approach.Ourresults on a large-scale analysis indicate that the pathway abundances provide a good summary of the functional capacity of a microbial community, well-suitable for the identification of relevant metabolic differences between distinct microbial communities.

P10
A pipeline for the analysis of MDA-based metagenomics read sets

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Introduction
Multiple Displacement Amplication [1](MDA, for short) is a powerful tool for sequencing samples from which only low amounts of DNA can be obtained. It has found applications in soil metagenomics projects of environments that are difficult to reach, such as mountain peaks [2] and deep-sea sediments [3].

Objectives
Unfortunately, MDA leads to coverage biases with a non-eglegible effect on downstream analysis results [4]. Our goal was to reduce the effects of the MDA-related bias in the computational analysis.

Materials and Methods
Our analysis pipeline first addresses the potentially uneven coverage in the upstream processing steps (Fig. 1A), consisting of clustering, assembly and gene prediction. The downstream analysis (Fig. 1B) is based on alignment of the ORFs to NCBI-NR and assignment of taxonomy and function using MEGAN [5]. Although MEGAN is usually used as an interactive graphical tool, we were able to run it in batch mode in our command line-based pipeline, employing an headless X-Server and TCL-Expect scripts. Finally, the assignments results are further characterized by employing statistical analysis tools.

Results
We developed a pipeline for the analysis of MDA-treated metagenomic samples which aims at reducing the effects of possible coverage bias. To demonstrate its usefulness, we applied the pipeline to publicly available MDA-treated datasets.

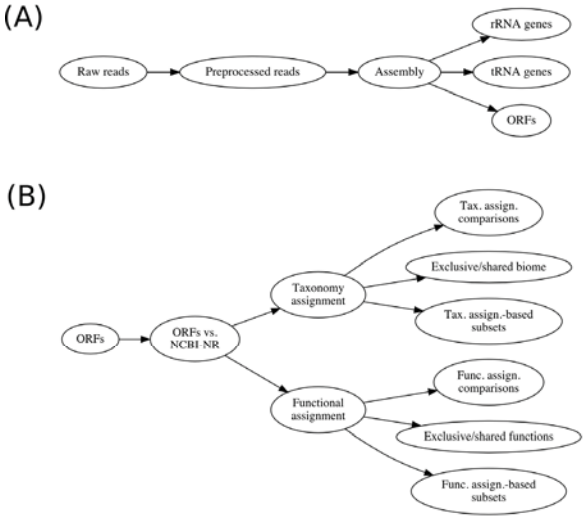

Figure 1: Upstream (A) and downstream (B) analysis.

Conclusion
The concerns regarding the MDA-treatment in metagenomics analysis can be reduced by accurately designing the analysis pipeline, taking the potential biases into account.

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P11
Extracting Genomes from Metagenomes, a study on permafrost soils in Barrow, Alaska

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While the majority of soil biodiversity remains un-discovered a newly developed bioinformatical approach on metagenomic data is being used to obtain whole genome sequences of microorganisms in permafrost soils. Permafrost contains an amount of green house gases equivalent to the amount found in the atmosphere and terrestrial plants combined, making this environment relevant for the understanding of climate change developments. Microorganisms found in permafrost soils are the key to understanding the mechanisms of gas exchange in the thawing permafrost. Paired-end Illumina datasets from the NGEE study [1] were assembled using the idba_ud assembler optimized for large datasets with uneven coverage [2]. An in-house pipeline for comparing similar metagenomic samples to obtain whole genomes of potentially unknown organisms is being tested on the dataset. By use of MetaGeneMark [3] and BWA alignment [4] for gene calling and subsequently an in-house developed assessment of abundances, sequences, which belong to the same genome, are clustered (unpublished data). Assemblies showed consistency in quality being dependent on the amount of sequencing data [figure 1]. With the fast expanding amount of metagenomic data available it is possible to extract ever more biological information by using newly developed bioinformatical tools.

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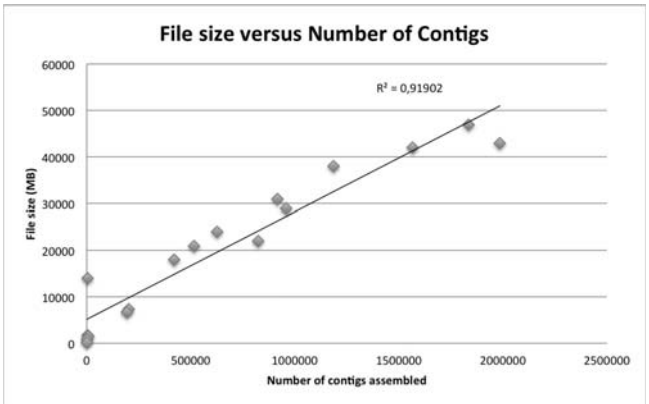

Figure 1. raw read data file size as a function of numbers of contigs that were assembled with IDBA_UD assembly platform.

P12
Metagenomic approach applied to the microbial community of a saline soil with high spatial variability

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Capturing the heterogeneity and the dynamics of complex soil microbial communities over time and in their spatial distribution represent a challenge in metagenomic analyses. The present study was focused on soil microbial DNA extraction and metagenomic DNA methods performance, from an area where some ecological variables acted as strong discriminant factors in fungal and bacterial spatial distribution.

A salt-affected soil was studied to evaluate the relationships between microbial community structure, soil features and DNA extraction effectiveness. 16 soils samples from A horizons were collected according to a random simple sampling scheme. DNA was extracted from fresh soil samples in triplicate with MoBio Power soil DNA extraction kit. Genereleaser [R] was used to further purify the extracts. DNA crude extracts were pooled in a single tube and then concentrations were calculated by means of Qubit® 2.0 Fluorometer. Bacterial, archaeal, and fungal communities were characterized by their 16S rDNA genes with T-RFLP method. Pyrosequencing-based analysis of the V2-V3 16S rRNA gene region, to identify changes in bacterial diversity and community structure was performed by means of 454 massive sequencing approach. Chemical and physical soil analyses were also performed.

The choice of DNA extraction and purification method had a significant effect upon bacterial, archaeal, and fungal molecular characterization from soil. The different concentration of salt, and calcium sulfate in soil influenced the structure and distribution of the microbial community even when comparing neighboring areas. Salts affected also the DNA extraction procedures.

The main compromise in ecological studies is between the need for a standardized protocol, and the natural variability of field parameters. In the present study, soil salinity represented both a natural gradient that defined the structure and distribution of microbial species in the environment, but also a critical element that introduced variability in the yield and quality of DNA extraction and purification. Robust techniques that allow the evaluation of the effects of environmental matrix on DNA representativeness must be set up.

P13
Vascular wilt in lamb´s lettuce: Metagenomic analysis of soil microbiota to identify relevant organisms

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Introduction
Since several years the occurrence of a symptom called “Gelbe Welke” (Vascular wilt) is an increasing problem during the cultivation of lamb´s lettuce (*Valerianella locusta*). At the beginning of cultivation the plants develop normally, but about two weeks after planting respective plants develop a reduced root system followed by typical wilt symptoms like yellow, chlorotic and limp leaves. Because of root reduction and decreased photosynthesis the plants are significantly smaller at the end of the cultivation period and are not marketable.

Objectives
Initial biotests have shown that the putative causal agent of the wilt symptoms is located in the soil. Using 454 pyrosequencing, composition of the micro biocoenosis in soils with symptomatic and asyptomatic plants were performed. The purpose of this study was to gain insights into the microbial community of respective soils and lamb´s lettuce rhizospheres, whereby rhizosphere is defined as the portion of soil adjacent to and influenced by the plant root.

Materials and Methods
Soil samples were taken at a depth of 0 - 15 cm with a soil sampler. Because lamb´s lettuce is cultivated in press pots the substrate of these press pots and the rooted soil underneath them were collected for rhizosphere analysis. The samples were homogenized and the total community DNA of two replicates was extracted using the PowerSoil® Isolation Kit. Both DNA replicates were combined in a pooled sample and the DNA was used for PCR with specific primers for the bacterial 16S rRNA region and the fungal ITS 1 and 2 region. The products were purified and submitted to tag-encoded 454 pyrosequencing at LGC Genomics GmbH. Obtained sequence data were evaluated using taxonomy-dependent analysis in MEGAN and OTU-based analysis in the automated pipeline CLoVR.

Results
454 pyrosequencing shed light on composition of microbial communities in soil samples and rhizosphere of lamb's lettuce plants and will help to identify putative causal agents of Vascular wilt.

Conclusions
Next generation sequencing technology provides a powerful method that can be used for large scale analysis of the composition of the micro biocoenosis of soil and rhizosphere.

P14
Functional screening of a metagenomic library for aerobic toluene degradation genes

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Introduction
Metagenomic analysis of polluted environmental sites facilitates the discovery of gene sequences from the little characterized or totally unknown microorganisms. It improves the design of novel specialized strains and enzymes useful for bioremediation. In metagenomics, functional screening (FS) is defined as library screening based on expression of an environmental DNA within the host. Detection of clones of interest is based usually on the detection of (i) produced metabolites and (ii) enzymatic activities in selective media. Unlike genetic screening, FS has greater potential to discover novel genes associated with an activity since it is not biased by a prior knowledge of genetic sequences. Successful FS depends strongly on the ability of environmental DNA to be recognized by the intrinsic host expression factors.

Objectives
1. Screening of a metagenomic library hosted into a mutant *Cupriavidus metallidurans* CH34 for aerobic toluene degradation.

2. Bioinformatic analysis of inserts from clones which use toluene as carbon source.

Materials and Methods
Cupriavidus metallidurans CH34 is a strain adapted to survive several forms of heavy metal stress and able to degrade toluene. In order to use it as an alternative host, its toluene monoxygenase operon was knocked out (deletion of genes encoding for toluene monooxygenase enzyme). The developed CH34 mutant had lost its ability to use toluene as it only source of carbon. A shuttle BAC vector (Bacterial Artificial Chromosome) (Lucigen, USA) was used to construct a large insert library from high molecular weight DNA purified from a former gasworks site located in Düsseldorf-Flingern, Germany. FS of the generated library was based on detection of transformant clones growing on minimal medium with toluene as the only carbon source. These clones were selected for Illumina sequencing and sequence analysis.

Results
A total of 28 clones were obtained after spreading the library on minimal medium with toluene as the only carbon source. These BAC clones harboured DNA fragments which complemented the truncated operon in CH34. Their sequences analysis is in progress.

Conclusion
Functional screening of a metagenomic library from sediment contaminated with monoaromatic hydrocarbons revealed that 28 BAC clones had putative toluene monoxygenase encoding genes. Given that we screened big insert library, new organization of monooxygenase genes as well as new enzymes involved in toluene degradation might be revealed.

P16
Simultaneous DNA, RNA, and protein extraction from a well-characterized soil for genomic and proteomic applications

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Reliable sample preparation is the first step towards generating high quality genomic and proteomic data. Methods of extraction and purification of DNA, RNA, and protein often focus on one biopolymer so that different kits or protocols are used, making it difficult to correlate information from the same sample. DNA and RNA purification from soil has been standardized and are easily co-extracted but protein extraction has proven to be more difficult. The more successful methods for protein extraction involve harsh conditions such as boiling and the use of SDS, NaOH, or phenol. Unfortunately, these methods are also efficient at co-extraction of contaminants and often lead to protein degradation and loss during processing, impacting protein analysis. Additionally, those methods are not easily adapted for DNA and RNA co-extraction. A validated DNA extraction method, which utilizes bead beating, was modified to co-extract RNA and protein from a well-characterized soil sample in triplicate. Nucleic acids were analyzed using next generation sequencing and protein was analyzed using 1D LC-MS/MS analysis. Results showed the successful determination of the relative abundance and diversity of the soil microbes for all three approaches utilized. Modification of an established DNA extraction method, allowed co-extraction of RNA and protein from the same sample with less co-extraction of contaminants, resulting in a less biased approach to sample processing and analysis using next generation sequencing and mass spectrometry based proteomics analysis.

P17

Microbial community potentially responsible for acid and metal release from an Ostrobothnian acid sulfate soil

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Soils containing an approximately equal mixture of metastable iron sulfides and pyrite are termed 'potential acid sulfate soil (PASS) materials'. Oxidation reactions occur when the iron sulfides are exposed to air and the subsequent acid and metal release causes environmental damage. Although acid and metal release from sulfide materials can be catalyzed by acidophilic microorganisms, the microbiology of acid sulfate soil materials is still unclear. This study contains two parts. The first part reports physical and chemical characteristics in the soil from the Risöfladan experimental field in Finland that has been used as agricultural land for more than 40 years; it identifies the microbial community in boreal PASS and 'acid sulfate soil' (ASS) direct both from the environment and after using the enriched acidophilic microorganisms at low pH; and lastly it investigates accelerated acid and metal release from PASS with an ASS microbial enrichment culture. The second part investigates how to mitigate the oxidation of sulfides and the formation of sulfuric acid on farmlands and how the chemical characteristics and the molecular phylogeny changes after treating the PASS with $CaCO_3$ and $Ca(OH)_2$ suspensions. Metal and acidity release from PASS was investigated by bioleaching experiment and the molecular phylogenetic analysis was based on restriction fragment length polymorphism (RFLP) patterns. Several known acidophilic microorganisms and environmental clones previously identified from acid- and metal-contaminated environments were identified in a depth profile through the plough and oxidized ASS layers. In addition, 16S rRNA gene sequences similar to sequences previously identified from cold environments were found. Metal release was accelerated by leaching of the metastable iron sulfides and pyrite in the presence of microorganisms enriched at low pH, suggesting microbes able to catalyze metal sulfide oxidation were present. The 16S rRNA gene analysis indicated the presence of strains similar to *Acidocella* sp. and other clones identified from acid mine environments. These data support that indigenous microorganisms adapted to low pH catalyze acid and metal release from ASSs and the influence of PASS chemical treatments to mitigate acid and metal release will also be evaluated.

P18

Beyond the soil dirt: Optimizing a RNA-based approach to study microbial communities

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RNA-based approaches are powerful and recent tools in the field of microbial ecology, giving the possibility to investigate the active part of microbial communities at a specific moment by amplicon sequencing of the 16S rRNA and ITS pools, and the functions expressed by the community by shotgun sequencing of mRNA. These approaches can reveal the effects of different environmental and anthropogenic stresses on the active part of the soil microbial community. However, working with RNA from environmental samples is still a delicate task, hampered by many practical challenges, and often requiring extensive technical tuning.

In this study, the effect of long term exposure to copper contamination on the activity of a soil microbial community was investigated through both sequencing of mRNA and amplicon sequencing of prokaryotic 16S rRNA and fungal ITS2. To do so, validation of the soil RNA extraction procedure was carried out. For that purpose, soil samples from three different levels of copper contamination (up to 3000 mg Cu kg^{-1} soil) were used to optimize the key steps involved in the RNA processing pipeline, including sample preservation, extraction, purification and mRNA enrichment.

Despite a clear negative correlation between extraction yields and copper contamination, RNA was successfully recovered from all samples. An increasing inhibition of the DNase treatment was also noticed concomitantly to copper levels. However, the DNase treatment was significantly improved by increasing the incubation time at 37°C from 30 min to 1 h, with frequent mixing and double amount of enzyme. Successful amplification was obtained from the highest copper contaminated samples by reducing the amount of total RNA used (e.g. 0.5-2.5 ng per reaction). Reverse transcription products generated from contaminated samples could be amplified only after dilution.

We conclude that both soil constitution and history are relevant factors for the success of the RNA extraction pipeline, being associated to soil sampling protocols, RNase free handling in the lab and downstream enzymatic processing. Incubation times as well as initial RNA concentrations and dilutions are also key factors to consider before undertaking RNA-based studies.

P20

The compound G2 increases yield of DNA extraction from clayey sediments up to 40X

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The compound G2 increases yield of DNA extraction from clayey sediments up to 40X

Introduction

Although the field of soil metagenomics has advanced and matured rapidly, the obstacle of retrieving sufficient amounts of DNA from certain soil types remains. Specifically, low-biomass clayey sediments provide a challenge for DNA extraction, as the phosphate-rich DNA backbone can bind strongly to clay particles in the soil. The compound G2 was developed at our laboratory, aimed at saturation of DNA binding-sites prior to cell lysis during soil DNA extraction.

Objectives

The aim of this study was to develop and test the additive compound G2 for increased yields of DNA during extraction from clayey sediments.

Methods
Uniform kits for DNA extraction with G2 was sent to 12 laboratories, along with a sample of clayey soil. DNA was extracted from the test soil, with and without G2 addition in the extraction procedure, by the laboratories and was returned to our laboratory for comparison of DNA yields. The bacterial 16S gene was quantified in the extractions using qPCR. The test laboratories also had the option to extract DNA from their own samples with the addition of G2 and shipping the DNA to us for qPCR.

A protocol for performing quality control of G2 batches was produced which also enables testing the DNA-sorptive potential of soils, through the addition of radioactively labeled *E. coli* and subsequent DNA extraction from a clayey sediment.

Results
The ring test showed that adding G2 increases the DNA yield up to 40X, although a high variance among replicates, probably inherent to soil heterogeneity, not always allows for differences that are statistically significant (P=0.05). Testing the other samples that were sent from ring test laboratories showed that G2 does not increase yields from all sample types, however, some did see an increase in DNA concentrations.

Conclusion
The G2 compound increases the DNA yield from certain clayey sediments and is easily applicable to the extraction procedure, regardless of preferred method.

P21
Using functional metagenomics for the discovery of new antimicrobial compounds

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Clinicians have predicted that the antibiotics currently used in hospitals, and required for most operations and treating infections, will no longer be effective within five to eight years given the rate at which resistance is developing. The aim of this project is to discover novel antimicrobial compounds using functional metagenomics to exploit soils from extreme or rare habitats such as the Antarctic.

Functional metagenomic libraries were constructed using methods as published (Brady 2007). Libraries were screened for polyketide synthase genes (PKS), and non-ribosomal peptide synthetase genes (NRPS), both of which are involved in the biosynthesis of secondary metabolites. PCR positives were subsequently sequenced and expressed in a heterologous *Streptomyces coelicolor* expression host. Chemical structure was elucidated using Liquid chromatography-mass spectrometry (LCMS) and Nuclear magnetic resonance (NMR). Soil function and structure was analysed using 454 pyrosequencing of PKS, NRPS and 16S rRNA genes.

Several hits were found for PKS and NRPS genes, to date two fosmids containing novel PKS and NRPS gene clusters have been fully sequenced. AntiSMASH was used to predict the compound structure encoded by these gene structures using their DNA sequence. The initial pEpiFOS-5 vector containing the cluster was modified using the PCR redirect system and moved into a modified *Streptomyces coelicolor* superhost for overexpression. Phylogenetic trees demonstrating the PKS and NRPS diversity were constructed for each of the soils analysed.

This study has produced several gene clusters producing compounds which can be exploited for their antimicrobial potential. Furthermore function and structure studies have identified the key characteristics of soil communities which are best for yielding the highest hit rate for PKS and NRPS gene clusters.

Reference
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P22
Fungal specific response in decomposing sugarcane leaf litter to no-tillage and bagasse mulching practices determined by Ion torrent ITS amplicon sequencing

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Soil fungi are the predominant decomposers of soil organic matter (SOM). To manage SOM in tropical agricultural soils, it is important to understand the effects of agricultural management on fungal communities and their decomposition of organic matter.

Our study site was located in a sugarcane plantation in LampungProvince, Sumatra, Indonesia. The goal of this study was to investigate fungal specific response in decomposing sugarcane leaf litter to no-tillage and bagasse mulch by using Ion Torrent's Personal Genome Machine (PGM). Our results showed that the most abundant fungal phylum was Ascomycota in all treatment plots, and Sordariomyctes were most abundant class found in the Ascomycota. However, the proportion of Basidiomycota was increased by bagasse mulch ($P < 0.01$) and Agaricomycetes were the most abundant class found in the Basidiomycota. On the other hand, Fungi incertae sedis were positively affected by no-tillage ($P < 0.05$), and Glomeromycota were marginally increased by no-tillage ($P = 0.07$). In class level, Cystobasidiomycetes of Basidiomycota, Dothideomycetes and Orbiliomycetes of Ascomycota were positively affected by no-tillage ($P < 0.05$). In addition, Mitosporic Ascomycota were marginally increased by no-tillage ($P = 0.06$). On the other hand, Sordariomycetes of Ascomycota were affected negatively by bagasse mulch ($P <0.01$) and there was an interaction effect of tillage × mulch ($P < 0.01$): the negative effect of bagasse mulch on this fungi was detected only under conventional-tillage system.

Overall, the result of high-throughput sequencing demonstrated that bagasse mulch altered dominant fungal taxonomic groups and inhibit some groups, whereas conversion from conventional tillage to no-tillage management increased the number of fungal taxonomic groups in decomposing leaf litter.

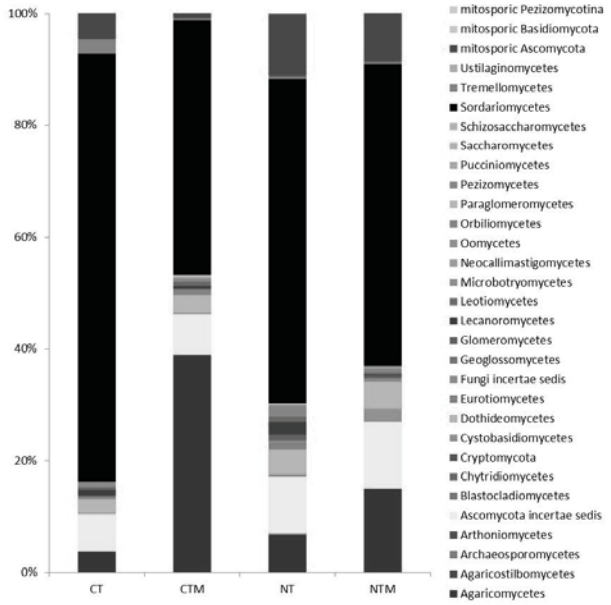

Fig. 1. Relative abundance of fungal class in sugarcane leaf litter from conventional tillage (CT), conventional tillage with bagasse mulch (CTM), no-tillage (NT) and no-tillage with bagasse mulch (CTM).

P23

Four years of experimental climate change modifies the community structure of denitrifiers and the related microbial drivers of N_2O fluxes in an upland grassland ecosystem

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Emissions of the trace gas nitrous oxide (N_2O) play an important role for the greenhouse effect and stratospheric ozone depletion, but the impacts of climate change on community structure of denitrifiers and the underlying microbial drivers of N_2O fluxes remain unclear. The aim of this study was to determine the effects of sustained climate change on field community structure of denitrifiers and associated N_2O fluxes, microbial enzymatic activities, and microbial population abundance in an extensively managed, upland grassland. We simulated global warming effect by exposing a grassland for 4 years to elevated atmospheric CO_2 (+200 ppm), elevated temperature (+3.5 °C) and reduction of summer precipitations (-20%) as part of a long-term, multifactor climate change experiment. While recording N_2O fluxes, potential nitrification and denitrification, microbial population size involved in these processes, we assessed the community structure of nitrite reducers (*nir*K) that perform the first step of denitrification. Our results showed that specific lineages of *nir*K denitrifier communities responded significantly to temperature. In addition, *nir*K community composition showed significant changes in response to drought. Both warming and simultaneous application of warming, summer drought and elevated CO_2 had a positive effect on N_2O fluxes, nitrification, N_2O release by denitrification and the population size of N_2O reducers and NH_4 oxidizers. *In situ* N_2O fluxes showed a stronger correlation with microbial population size under warmed conditions compared with the control site. Path analysis explained more than 85% of *in situ* N_2O fluxes variance by specific denitrifying lineages, soil temperature and denitrification activity. Overall, our study underlines that climate-induced changes in grassland N_2O emissions reflect climate-induced changes in microbial community structure, with a potential selection of more adapted types. These in turn may modify microbial processes.

P24

Influence of tillage and fertilizer type on N cycling microbial populations

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No-tillage as soil management method is increasingly used in conventional agricultural practice due to its beneficial effects on soil properties. As organic agricultural practice abstains from herbicide use, reduced tillage seems to be an adequate analog to reduce weed pressure, while preserving soil fertility and preventing soil degradation. It is known that reduced tillage increases topsoil C stocks, restricting N availability and, consequently, N transformation processes. The type of N addition used as fertilizer determines prevailing N transformation pathways in agriculturally managed soils. Within soils N budget, the emission of N_2O is of key importance owing to its properties as greenhouse gas and its long atmospheric lifetime. As cycling of N in soils is almost entirely controlled by microbial communities it is crucial to understand microbial responses on diverse N additions and their function regarding N_2O forming and reducing processes under conventional till and reduced till soil cultivation. In this study soil samples from different depths (0-10cm vs. 10-20cm) and tillage systems (conventional vs. reduced till) were incubated in a laboratory experiment under near field conditions. The impact of different N fertilizer types (Cattle slurry, mineral fertilizer and cattle manure compost) on N_2O emissions was assessed in addition to other geochemical parameters (CO_2, CH_4, NH_4^+, NO_3^-, DOC and pH). In order to investigate microbial responses on N addition, functional groups of nitrifiers and N_2O reducers were quantified by targeting the functional genes *amoA* (*Archaea* and *Bacteria*) and *nosZ* via a qPCR approach. First results show significant changes in ammonium oxidizing archaea and *nosZ* containing bacteria between tillage systems. Furthermore, addition of cattle slurry promotes growth of N transforming microbial populations under conventional tillage, compared to other fertilizer treatments.

P25

Agricultural impacts upon the soil microbiome:the implications on carbon cycling.

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Introduction

Global agricultural practices utilize fertilizers so as to maximise outputs. Sulphur is one such fertilizer used to maximise crop growth [1]. The addition of fertilizers may lead to a shift in the soil microbiome which may ultimately influence the cycling of atmospheric carbon [2]. This coupled to unknown levels of atmospheric carbon which may be transformed into soil carbon [3], may have a global impact on climate change and soil carbon stocks.

Objectives
We have previously demonstrated the implementation of an environmental chamber in the monitoring of an atmospheric carbon flux within a 13C isotopically enriched soil [3]. Here we aim to measure any changes in natural soil microbiome associated with the addition of sulphur and link shifts in the soil microbiome to the cycling of atmospheric carbon and ultimately the formation of soil carbon.

Materials and Methods
In this work we utilise stable isotope probing (SIP), PLFA analysis, 16s rRNA 454 pyrosequencing and NMR analysis in order to track the fate of CO_2 in a microbial environment and, ultimately, identify the main microorganisms actively participating in the uptake of and transformation of atmospheric carbon.

Results
We discovered that certain chemoautotrophic microorganism DNA, affiliated with β-Proteobacteria, became labelled following incubation within a $^{13}CO_2$ environment. Microbial lipid labelling revealed the impact of sulphur addition upon chemoautotrophic processes and NMR analysis revealed the ultimate fate of CO_2 in the natural environment.

Conclusion
As one of the major global industries it is no surprise that the agriculture industry impacts upon our environment. However, careful agricultural practices may hold the potential to alleviate ever rising atmospheric carbon levels and also improve end product output.

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P26
Effects of N deposition on diazotrophic activity and distribution of microorganisms associated with *Sphagnum magellanicum*

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Introduction
Sphagnum-dominated peatlands are important carbon (C) sinks and greenhouse gas filters[1]. These ecosystems are generally N-limited, but increasing levels of atmospheric N-deposition due to anthropogenic activities, have the potential to shift these systems from C-sinks to C-sources[2]. N-deposition effects on plant communities have been well studied, but little is known about the effects on the N-fixing microbial community associated with *Sphagnum* spp. in peatlands[3].

Objectives
We aim to quantify the short-term and 'memory' effects of high N-deposition on diazotrophic activity and community associated with *Sphagnum magellanicum* in ombrotrophic bogs. Ultimately we want to understand the influence of N-deposition on ecosystem functioning

Materials and Methods
N_2 fixation rates associated with *S. magellanicum* from pristine and high-N-deposition sites were measured by acetylene reduction assays, calibrated with $^{15}N_2$ (with and without C_2H_2). DNA was extracted from the same samples and diazotrophic community diversity was analysed by clone library construction of nifH genes and by amplicon sequencing of nifH genes (Ion Torrent PGM™).

Results and Conclusion
N-fertilisation had a negative effect on N_2 fixation rates in mosses from both pristine sites and areas with high N-deposition. Labelling showed that acetylene decreased $^{15}N_2$ uptake, questioning the usefulness of this commonly method. Community diversity analyses showed a diverse diazotrophic community, of which the majority of nifH sequences belonged to a cluster without cultured representatives and unclear affiliation.

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P27
Dominant bacteria are responsive to 20+ years of experimental soil warming in a mixed deciduous forest

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Introduction
Heterotrophic soil respiration increases with temperature in the short run, but longer-term responses are often mixed. After 20+ years of experimental 5°C warming in mixed deciduous stands at the Harvard Forest, it is clear that purely kinetic or substrate availability effects cannot explain observed changes in respiration, and that adaptation of the bacterial community is likely to play a role. We aimed to determine how warming has affected the soil bacterial community across a chronosequence at this site.

Methods
In fall 2011, soil samples were collected from the mineral and organic horizon of replicated plots that had been warmed for 6, 9, or 21 years. We extracted the DNA using a modified CTAB procedure and performed Illumina MiSeq sequencing on the 16S rRNA genes. We filtered for quality then used the Qiime pipeline to pick OTUs, resulting in 1.56 million reads separated into 43,909 OTUs. We examined treatment effects on β-diversity using UniFrac, and completed edge PCA to identify phylogenetic groups responsible for differentiating between warmed and control plot samples. We used seven indicator species analyses to identify specific taxa representative of warmed or control soil communities. We also completed phylum-specific qPCR to validate the relative abundances.

Results
The warming treatment had a significant effect on community only in the organic horizon of the longest-running warming plot. In identifying the dominant taxa (relative abundance ≥0.1%), we found that just 155 OTUs accounted for more than half of the observations, and the most abundant of these were also some of the most responsive to warming treatment.

OTUs within Acidobacteria, Actinobacteria and α-proteobacteria comprised most of the dominant subset community, and a single

Actinomycete accounted for 10% of the observations. QPCR confirmed that α-proteobacteria dominate in these soils, especially under warming.

Conclusions
It took an extended period of warming for a shift in the bacterial community to appear, well after physiological effects (*i.e.* increased net respiration) were evident. Our data suggest that a relatively few taxa in an uneven community may be responsible for large changes in soil function, which seems counterintuitive considering the relatively high richness of soils. This finding, along with future studies on the behaviors of these putative keystone bacteria, are promising for modeling complex soil communities based on relatively abundant keystone species.

P28
Monocropping drives the homogenization of bacterial communities at a regional scale in no-till fields of Argentinean pampas

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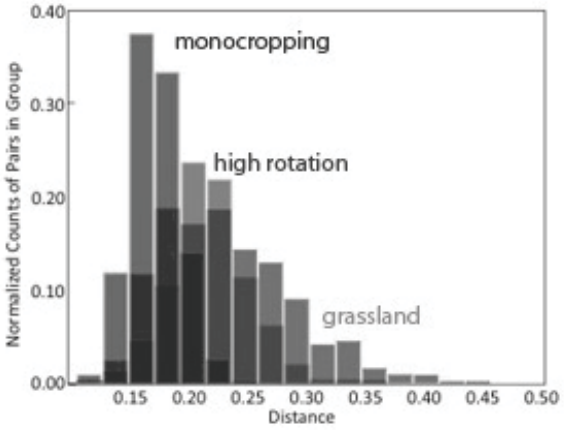
Introduction
Previous studies focusing on local diversity were unable to grasp any deleterious effects of monocropping on soil bacterial communities. In opposition, variation of beta-diversity at a regional scale can provide insights into the importance of habitat conditions in structuring the communities

Objectives
The aim of this work was to uncover the response of soil bacterial communities to monocropping in comparison to high crop rotation in no-till production fields

Methods
Blocks of treatments were sampled at 4 different locations across a scale of 400 km along 3 successive cropping seasons. In each location we sampled a natural field (grassland) and two production fields managed under no-till according to the following standards: 1) Crop rotations and appropriate nutrient amendment, 2) Soybean monoculture and minimal nutrient inputs. Three replicates from the top 10 cm of bulk soil separated 50 m from each other were taken for each treatment, site and time, making a total of 108 samples. The Mothur and QIIME pipelines were used to process and analyze the reads of the bar-coded 454 sequencing of the V4 region of the 16S rRNA. A total of 333180 OTUs averaging 254 bp were used for analysis

Results
There were no significant data differences in α-diversity related to sites or soil management. However, histograms of pairwise distances between all pairs of samples within treatments (weighted Unifrac, Fig 1) revealed that β-diversity was reduced and had narrower breadth in monocropping agriculture than in grassland soils while fields under crop rotations was in between. At the phylum level the magnitude of this effect depended on the richness of each taxon at the regional scale

Conclusions
Crop monoculture drives the homogenization of bulk soil bacterial communities across a regional scale. Variation in taxa responses to agricultural management could be explained by differences in gamma diversity



P29
Non-target effects of chemical v/s bio-pesticides on rhizospheric microbial diversity

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Introduction
Biopesticides are supposed to be an ecologically safe alternative to chemical pesticides that have been used extensively to produce disease-free crops. One of the major research gaps in this area includes the non-target effects these pesticides impose on soil microbial diversity apart from their target effects.

Objectives
The primary objective of this study was to investigate the effects of three chemical pesticides (chlorpyrifos, endosulfan and cypermethrin) and a biopesticide, azadirachtin, on microbial community diversity in *Vigna radiata* rhizosphere.

Materials and Methods
Both cultivation-dependent and independent methods have been used to study the effects of these pesticides on rhizospheric microbial population. Rhizospheric populations of specific groups were cultivated on selective media and colonies were counted. Universal bacterial primers (for 16S rRNA) were used to analyze the total bacterial community from DNA and RNA extracted from the system. Key players in the rhizosphere at different time points were identified through fingerprinting using denaturing gradient gel electrophoresis (DGGE).

Results
All these pesticides manifested initial inhibitory effects on several PGPR including *Pseudomonas* sp. and N fixing bacteria, followed by a significant increase at later stages. Also, the growth of phosphate solublizers was highly favored by chlorpyrifos and endosulfan while azadiractin adversly affected these microbes including *Bacillus* strains. DGGE profiles of resident and active bacterial population indicated that at higher doses, the effects of the biopesticide mimicked those of the chemical pesticides. The

results obtained were validated by replacing endosulfan with cypermethrin. It was found that cypermethrin continuosly suppressed the growth of phosphate solublizers, and *Pseudomonas* sp. while chlorpyrifos and azadiractin initially increased and then inhibited their growth. All these pesticides were observed to adversely affect the growth of N-fixing bacteria at the end point.

Conclusion
A dose- and time- dependent effect on active bacterial populations was observed by both azadirachtin and chlorpyrifos. The study also highlights the non-target effects of both chemical- and bio-pesticides on active microbial populations, thereby raising the issue of safety of biopesticide's use in agriculture as an alternative to chemical pesticides.

P30
Impact of agricultural practices on soil microbial communities in a loamy soil in Belgium

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The use of fertilizers in agricultural soils is becoming a real environmental issue (an obvious example is eutrophication caused by leaching of phosphorus and nitrates). Much research has focused on finding ways to reduce the use of chemicals, and investigating microbial life may lead to solutions. We know that bacteria and fungi are deeply involved in nutrient cycles. Recently the emergence of massive parallel sequencing has enabled us to realize that microbial diversity is huger than we expected. With such a tool it should be possible to study how soil management practices affect the microbial diversity of agricultural soils. A few such studies have been conducted, most of them focusing on bacteria. For Belgium in particular, there is a lack of data on this topic. Here the aim was to see how residue management and tillage practices affect communities of both bacteria and fungi in Belgian agricultural soils. For this we used 454 pyrosequencing of 16S bacterial and 28S fungal rRNA genes. Soil samples came from an experiment in which faba beans were grown with four soil management practices (tillage and no tillage, with and without crop residues), each repeated four times in a Latin square. Several chemical and physical characteristics were measured on each sample. The experiment is ongoing and results will be presented at the meeting.

P31
Plant species composition and season influence shifts on the soil microbial community composition in response to climate change

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Recent studies showed that temporal and spatial effects of climate change operate at species and community scales by shifting their climatic niche. Although a number of studies reported drought as driver of changes in microbial community composition, we do not yet know the causal relationship between the change in the plant and microbial community composition.

In this study we used two sets of 32 perennial plant species, 8 species each, composed of grasses, legumes, small herbs and tall herbs. Species pooling was based on their distribution along Europe. The first set includes plant species which are predominantly distributed in South-West Europe (SW) whereas the second set includes plant species mainly distributed in North-East Europe (NE) representing potential winners (positive species) and losers (negative species) of the predicted climate change respectively. The experimental setup includes drought and plant species origin treatments. (1) Drought treatments: ambient precipitation and simulated drought period via roofing where the simulated drought was done in June and September to assess temporal variations. (2) Plant species origin treatments: positive species, negative species and a mixture of positive and negative species, where each plot contained 16 random species composed of four species per functional group. The experiment was set in April 2010 including control plots without plants. After two years of drought manipulation, composite soil samples were collected in June and September 2012. The microbial community was assessed using pyrotag sequencing of 16S rRNA.

We found a shift in the microbial community composition corresponding to other soil properties. The simulated drought, plant species origin, and season were found as the major drivers of the observed shift in the microbial community composition. Apart from the consistent impact of drought on the microbial community composition in both seasons, we also found a shift in the composition of the dominant bacterial communities in June and September. This implies seasonal variation seasonal variation of the microbial community composition even under drought condition. The implication of these results in predicting changes in microbial community and their functional diversity in responses to climate change will be discussed.

P32
Effect of long term differential fertilization on the soil microbial eukaryotic community: a metagenomic analysis

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Soil organic and inorganic fertilizations have been commonly used since more than one century to increase yield of crop fields. Although their effect on plant growth through increase in nutrient availability is obvious, little is known about their impact on belowground microorganisms, particularly on a long term scale. Eukaryotic microbes, which carry as diverse functions as primary production (e.g. microalgae), predation (e.g. protists) or decomposition (e.g. yeast), are key components in the soil food web and nutrient cycles. As fertilization induces changes in natural

nutrient cycles, important modifications in soil communities have to be expected in intensely fertilized soils.

In order to understand the effect of fertilization on the soil eukaryotic microbial community at a finer scale, a multiple barcoding approach was applied on soil coming from the four extreme treatments of the Bad Lauchstädt Long Term Static Fertilization Experiment (Germany).

Soil genomic DNA was parrallely PCR amplified by a general eukaryotic 18S rRNA primer pair as well as with specific primer pairs for protists (18S rRNA; Cercozoa, chrysophytes, kinetoplastids), Fungi (internal transcribed spacer, ITS), and algae (universal plastid amplicon, UPA), and sequenced on a Roche GS FLX 454 automated pyrosequencer.

Low variations in OTU richness were observed between the different fertilization treatments, which was partially explained by a high proportion of OTUs belonging to the core microbiome (i.e. OTUs shared between all fertilization treatments, from 46 % of OTU in eukaryotic dataset to 77 % in chrysophytes dataset). However, OTU community compositions appeared highly structured in response to organic fertilization (PERMANOVA p.values < 0.05) in all datasets (specific primer pairs) but not in the general eukaryotic 18S dataset. Similarly, phylogenetical diversity was significantly influenced by the organic fertilization in all datasets apart chrysophytes dataset,whereas mineral fertilization only played a minor role. A network analyze additionnaly showed that non-random co-occurences were only found between OTUs predominantely occuring in organic fertilized samples or predominantely occuring in non-organic fertilized samples.

In this study, we showed that eukaryotic microbial community significantly changed at all trophic levels due to an increase in organic nutrients availability. We also observed that such environmental shaping of OTU community composition can be missed when using a general eukaryotic marker.

P33

Changes in bacterial community structure as a response to drought stress in forest ecosystems - a roof experiment.

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Climate change is predicted to severely affect precipitation patterns across central Europe. As microbial communities perform many functions in soil and plant-microbe interactions, it is important for our understanding of climate- ecosystems feedbacks, to know how microbial communities will response to drought stress in forest ecosystems.The aim of our study is to assess the effects of the reduction in precipitation on the linkages between understory vegetation and soil microbial community. For this purpose, we induced drought across a range of forests at different sites (Schäbische Alb, Hainich and Schorfheide) and land-use intensities of the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/1/home/).We used T-RFLP analysis of the 16S rDNA gene to analyse the total and the metabolically active (RNA-based) bacterial community structure in nine different forest plots located in three different landscape areas (sites). The pre-drought fingerprints showed significant differences between the sites and nearly all plots and we detected no significant differences between control and pre-roofed subplots. After five month of drought treatment, several significant differences between the roof and control plots were visible. Based on these results, a detailed analysis of the metabolically active bacterial community was performed by 454 pyrotag sequencing after five months of drought. A total of 622,000 sequences could be obtained which displayed major differences at the genus or family level. Though main impact on the soil bacterial community was due to the sites and land use intensity (including the varying soil characteristics), significant effects of the drought treatment could be revealed. Moreover, the strength of the drought effect differed between the different plots and seems to depend on specific environmental conditions on the respective plot.

P34

Infection dynamics of insecticide-degrading symbionts in the bean bug *Riptortus pedestris*: Which bacteria dominate in insecticide-sprayed soil and then infect to the bugs?

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Fenitrothion(*O*,*O*-dimethyl*O*-(3-methyl-p-nitrophenyl) phosphorothionate, or MEP) is a widely-used organophosphorus insecticide and reported to be degraded by soil microbes. Recently, we discovered a novel MEP-resistance mechanism in insects: the bean bug *Riptortus pedestris* becomes MEP-resistant owing to the symbiosis with a MEP-degrading *Burkholderia* in the gut. We also reported that the bean bugs acquired their symbiotic bacteria from ambient soils in their nymphal stage. However, little is known about the infection dynamics of MEP-degrading bacteria from soil to insects.

To reveal the infection dynamics, here we investigated the structural transition of soil microbiota after MEP-spraying and determined MEP-degrading symbionts acquired by the bean bugs from the MEP-sprayed soil.

Soils collected from agricultural fields were set into plant pots and incubated with weekly spraying of MEP. As a control, distilled water was sprayed. Soil samples collected at two-week intervals were used for isolation of MEP degrading bacteria and Illumina sequencing of bacterial 16S rRNA gene. In addition, *R. pedestris* were reared on each of the collected soils from hatch to adulthood. Symbiotic gut organs dissected from the adults were subjected to MEP-degrading activity assay, isolation of symbiotic bacteria, and Illumina sequencing analysis.

After MEP-spraying, MEP-degrading bacteria in the soil drastically increased, most of which were members of the genus *Burkholderia* and *Ralstonia*, The cell density of MEP degraders in the soil was gradually raised as the number of spraying increased. Illumina sequencing of 16S rRNA gene confirmed the drastic change of *Burkholderia* density after MEP-spraying, compared with that in the control. Bean bugs infected with MEP-degrading bacteria were detected only when the insects were reared on the MEP-sprayed soil, and their infection rate gradually increased to 88.4±9.1% in accordance with the number of MEP-spraying. Phylogenetic analysis of the soil-derived and gut-associated *Burkholderia*

revealed that a number of gut-associated MEP-degraders dominated in the MEP-sprayed soil, highlighting the infection dynamics of MEP-degrading bacteria and key players both in soil environment and insect gut.

P35
Endophytic bacterial diversity in Eucalyptus and the influence of mixed plantation and N addition

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The planted forests represent a cheap and renewable source of raw material for industry, and reduce pressure on the native vegetation. The improvement of this system is of great interest. Endophytes have been characterized by its potential as plant-growth promoting bacteria (PGPB), representing an important source of biotechnological products. This work aimed to study the endophytic bacterial diversity in Eucalyptus and evaluates the effect of N addition by fertilizer or by intercropping with a legume tree (*Acacia mangium*). Samples of *E. urograndis* roots were sampled under three different treatments (ie, i. without N addition; ii. with N addition; iii. intercropped with *A. mangium*). The disinfected fragment roots were used for DNA extraction and subsequent 16S rDNA amplicon pyrosequencing. After all quality control procedures, 6225 sequences were obtained per sample, which were grouped in more than 1400 OTUs, classified into more than 350 different genera, being the most comprehensive diversity study of endophitic bacteria in tree species. To all treatments, Actinobacteria, Proteobacteria and Firmicutes were the most abundant phyla, respectively. The statistical analysis revealed a significant influence of treatment on the bacterial community structure, being *Burkholderia*, *Nocardia*, *Streptomyces* and *Staphylococcus* the more influenced genera from the most abundant.

Our results indicated a great abundance of known biological nitrogen fixing genera inside Eucalyptus roots, considered until now a plant with no association with nitrogen fixing bacteria, and that the addition of N or the use of mixed plantation with the legume plant *Acacia mangium* influence Eucalyptus endophytes bacterial diversit

P36
Plant coverage determine the fungal community structure and diversity in a forestry system

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Fungi are ubiquitous and important contributors to soil nutrient cycling with a vital role in C, N and P turnover, with some having direct beneficial relationships with plants. However, the knowledge about the factors that modulate the soil fungal community is still poorly understood. We studied to what degree the tree species affected soil fungal community structure and diversity by pyrosequencing the 28S rRNA gene in soil DNA, and whether intercropping could be used to manage for desired fungal species. More than 50,000 high quality sequences were analyzed from three treatments: monoculture of *Eucalyptus*; monoculture of *Acacia mangium;* and a mixed plantation with both species sampled 2 and 3 years after planting. We found that the plant had a major effect on soil fungal community structure, with the Eucalyptus soil having x% Basidos and y % Ascos, but that the Acacia soil had, k and l%, respectively. The intercropping of *Acacia mangium* in a *Eucalyptus* plantation significantly increased the number of genera of fungi, the diversity index and introduced or increased the frequency of several genera that could not be found in the monoculture cultivation. Our results suggest that the management of the soil fungi is possible by manipulating the plant composition, and intercropped systems can be a means to achieve that.

P37
Assessing nitrous oxide emissions and microbial structure after sugarcane fertilization

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Introduction
Sugarcane is a crop for bioenergy in Brazil and one of the main concerns in the production of this crop is the impact on the environment, in particular in greenhouse gases (GHG) emissions. Recent studies have shown that the N_2O emissions related to sugarcane production are dependent on soil management practices. The way to mitigate N_2O emissions would be to understanding the conditions and the processes involved in the N_2O production and consumption. However, no studies have linked the emissions of GHGs with soil-borne microbial communities, which are the main players in nutrient cycling.

Objectives
In this study we assess management practices for sugarcane production by comparing N_2O emissions and soil-borne microbial community structure using pyrosequencing data.

Materials and Methods
We have 5 fertilizers combinations applied on soil [Nitrogen control, NH_4NO_3, vinasse (bioethanol byproduct), NH_4NO_3+vinasse, concentrated vinasse] and these treatments combined or not with straw crop residues in the field (22°41'19.34"S; 47°38'41.97"W). Fertilization was carried out in November 2012 and since then the greenhouse gases were sampled until the harvest. During the first 60 days, 31 samplings were carried out to determine soil inorganic N, soil moisture and perform the DNA extraction (n=3). Based on the results of the N_2O emissions, 8 sampling dates were chosen for microbial molecular analyses by next generation sequencing. We use the 454 platform to assess the Archaea and Bacteria structure targeting 16S rDNA (V4 region, primers 515F and 806R; LIB-L kit for unidirectional sequencing). The microbial structure will be correlated with soil chemical factors and N_2O emissions in order to understand the microbial groups related with N_2O emissions.

Results
We verified a high N_2O fluxes range between the treatments (Fig. 1). Thus, we also expect notice those groups related with its production/consumption. The net emissions of N-N_2O (µg m^2) after

60 days from fertilization were 0.029 (concentrated vinasse); 0.015 (NH_4NO_3); 0.009 (control); 0.055 (NH_4NO_3+vinasse); and 0.015 (vinasse) from treatments with no straw maintenance. From the same treatments but with straw maintenance in the field the emissions were 0.033; 0.043; 0.009; 0.095; and 0.013, respectively. The sequences analysis from pyrosequencing are in progress (1371,699 reads; average length of 283 bases).

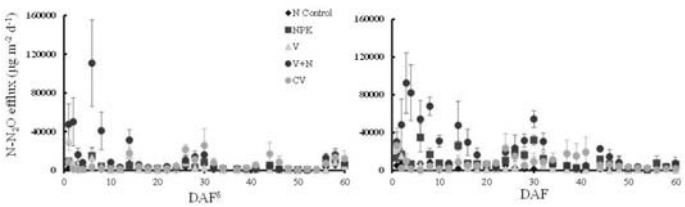

Fig. 1. Nitrous oxide fluxes from soil after fertilization. DAF - Days after fertilization.

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The Arctic snowpack microbial community highlighted by metagenomics and metatranscriptomics

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The Arctic seasonal snowpack can extend at times over a third of the Earth's land surface. This chemically dynamic environment constantly interacts with different environmental compartments and especially with soil. Some studies have focused on the impact of snow cover and melting on soil microbial community structure and function, such as nitrogen mineralization. However, the microbial community associated with the snow habitat itself and its potential role in biogeochemical cycling remains poorly understood. Previous studies based on 16S rRNA gene analysis revealed a high diversity of microorganisms within the snowpack. Here, we examined both the microbial community structure and function by applying a global approach using metagenomics and metatranscriptomics. From the 250 thousand sequence reads in all our snow metagenomes, the majority (between 58% and 88%) were unassigned to specific metabolic categories, which signals the apparent lack of related functional data in sequence databases. However, snow metagenome analyses demonstrated major shifts in function distribution during the season indicating that the snowpack is a dynamic ecosystem. These changes seem to be correlated to fluctuations in environmental conditions as some chemical parameters, like mercury or methyl-mercury concentrations, were varied with function. Comparing snow metagenomes with publically available datasets from different ecosystems including soil, we can describe the specific functional signature of the snowpack microbial community. Some functions like oxidative stress response or lipopolysaccharides biosynthesis are more highly represented in snow metagenomes than in those from other ecosystems. These functions are probably related to how microorganisms cope with the harsh conditions characteristic of the Arctic snowpack. The presence of mRNA in the Arctic snowpack further supports the hypothesis that microorganisms are metabolically active in the polar snow ecosystem. The expression pattern derived from the mRNA sequencing gives an indication of which microorganisms are active and their respective metabolic processes. Finally, this study could lead to a better understanding of how these two compartments interact and the impact of snow cover decrease for snow covered soil ecosystems.

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Unravelling keyplayer denitrifiers and dynamics of transcription activity in high and low pH soils

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Denitrification in soil is a major source of nitrous oxide (N_2O) emissions. Our recent study showed that the N_2O/N_2 product ratio is strongly dependent on soil pH. Transcription of genes coding for nitrite- and nitrous oxide reductases was detected during the first 5h of anoxic incubation. Regulation of denitrification differs between organisms and our results from pure culture studies demonstrate that some denitrifiers have a rapid, complete onset of all denitrification genes while others show progressive onset or onset by only a small fraction of the population. In complex communities this would result in successive onset of denitrification where the "early birds" induce transcription in others by intermediates (NO_2^-, NO).

Objectives

1) compare the phylogenetic composition of the denitrifier community in soils with contrasting pH

2) identify active groups of denitrifiers

3) determine whether there was a successive initiation different denitrifier populations

Microcosms were incubated in a robotized system that measures all denitrification intermediates. Samples were collected at 1h, 2h and 4h, guided by the gas kinetics to capture peaks of transcription. Functional genes and their transcripts (*napA, narG, qnorB, nirS, nirK, nosZ*) were amplified and sequenced.

In the pH 8.0 soil, microorganisms affiliated with the genus *Dechloromonas* expressed *nirS* at high levels (40-50% of the total cDNA library) at all three sampling times, while being less abundant in the DNA library (napA at increasing levels at the three time points (from 0% to 22%). Organisms related to *Ralstonia* had high *nirS* transcription at 1h (23%) and 2h (22%), then declined at 4h (9%). The *Pseudomonas* group, which only constituted 15% of the DNA, expressed high *nosZ* levels at 2h and 4h (44% and 45%). The *Herbaspirillum* group, in contrast, accounted for 58% of the *nosZ* DNA library, but 25% in the 2h cDNA library. *Rhizobium* was a predominant genus (46%) for the *nirK* gene, but no transcription was observed. Transcript analyses of the low pH soil were hampered due to methodological problems which are now resolved, and results are underway.

The results revealed a diverse denitrifier community, where different populations triggered gene transcription at different time points. The microorganisms related to *Dechloromonas, Ralstonia, Herbaspirillum* and *Pseudomonas* were identified as key denitrifiers. The dominance of various phylogenetic groups at different denitrification steps may indicate that truncated denitrification is a common phenomenon in this soil.

P40

Pesticides influence on bacterial communities from expired pesticides landfills

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Pesticides pollution is one of the greatest problems both in land and aquatic ecosystems. It is widely acknowledged that certain pesticides accumulate in the food chain and may exert negative impact on human health. We were interested in the impact of pesticides, at extremely high concentrations, on bacterial communities in soils.

To assess if pesticides change bacterial diversity and community structure, we isolated metagenomic DNA from samples collected from expired pesticdes landfills as well as control (uncontaminated) soils and pyrosequenced 16S rRNA gene fragment libraries amplified on metagenomic templates. The reads were processed with MOTHUR using PyroNoise and UCHIME for denoising and chimera detection, respectively. Shared OTU data were analyzed with the vegan R package.

We found that DDT was the only pesticide changing bacterial diversity and community structure, while other organochlorine ones changed only diversity. The presence of organochlorine compounds was correlated with most abundant OTUs, mainly belonging to genus *Pseudomonas.*

Bacterial communities do not seem to be significantly affected by pesticides with the exception of organochlorine ones.

P41

Cattle outdoor husbandry results in the introduction and survival of rumen-borne *Archaea* and *Bacteria* into the intensively pastured soil

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Introduction

Archaea and bacteria are important drivers for nutrient transformations in soil and significantly participate in production and consumption of important green house gases. In upland soils, considerable changes in soil functioning (enhanced methanogenesis and denitrification) occurred as a consequence of high external nutrient inputs, grazing and trampling activities associated with cattle outdoor husbandry during winter time, when animals are kept on a small area close to stables.

Objectives

We aimed to identify changes in archaeal and bacterial diversity and community structures in four grassland soils, being differently affected by cattle outdoor husbandry.

Materials and Methods

Two soils under actual impact of cattle during winter (3 years, BI and 17 years, SI), soil regenerating from cattle impact (MR), and control soil (CO) were sampled at Borova farm (Czech Republic). Cattle excrements (EX) as a source of allochthonous microbes were collected at the same area. To assess archaeal and bacterial community composition, the pyrosequencing-based analysis of 16S rRNA genes was employed.

Results

CO soil was dominated by Thaumarchaeota (82.1%), while SI soil was enriched by methanogenic Euryarchaeota (60.2%). In cattle affected soils, the presence of Methanobacteriaceae, Methanosarcinaceae, and Methanomicrobiaceae was observed, while hydrogenotrophic Methanocorpusculaceae typical for EX were not detected. Contrastingly, Methanosarcinaceae were not found in EX, while they were present in MR and SI soils. Bacterial community shift was observed in SI soil, characterized by the increase of Firmicutes, Actinobacteria, and Chloroflexi, and decrease of Acidobacteria and Alphaproteobacteria.

5. Conclusions. Archaeal and bacterial community structures were significantly affected by both outdoor cattle husbandry, and by 3 years of regeneration, mostly due to direct (introduction of fecal microbiota) and/or indirect (due to altered soil properties) effects. Firmicutes, Bacteroidetes, Methanobacteriaceae, and Methanomicrobiaceae indicated the introduction of fecal microbes into cattle affected soil and their successful establishment, while Chloroflexi and Methanosarcinaceae suggested the enrichment of soil-borne microbes in altered environmental conditions.

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P42

Predictive model of soil molecular microbial biomass by using the French soil quality monitoring network data.

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The soil is the support of the human construction and agricultural production. Overexploitation for the development of intensive agriculture and industrialization has led to a significant erosion of biodiversity, so it becomes urgent to provide users the means to evaluate soil biological status of their production support and the impact of related practices (agricultural, industrial, urban…).

Among the native soil organisms, microbial communities (bacteria and fungi) are the most important in density (10^6 to 10^9 individuals / g of soil), diversity (10^3 to 10^6 species / g soil) and also for their involvement in the biological functioning of soils. Therefore, the ecosystemic services provided by the soil will be strongly dependent on the level of abundance and diversity of indigenous microbial communities. In addition, as regard to the increasing demand from land users to characterize the biological status of their soil it is now urgent to develop operational expertise in the field of biological characterization of soils. Technical standards has been recently optimized in terms of characterization of density and taxonomic diversity of soil microbial community using molecular tools based on soil DNA extraction, but now mathematical interpretation and standards are needed to finalise the diagnosis.

In this context, we develop a polynomial parametric model allowing predicting microbial abundance and diversity in soil. This model is based on the exploitation of the MicroSol database © of the GenoSol platform (http://www2.dijon.inra.fr/plateforme_genosol) which hosts tens of thousands of data on microbial abundance and diversity in soil. Predictions are based on the main factors driving microbial community in soil (soil type, land use and climate). These models are the first developed in environmental microbiology to predict the range of natural variation in microbial community as well as according to land use.

P43
Amazonian deforestation alters bacterial networks in soil

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Face to global demand for food, fiber, and biofuels, Brazil is quickly surpassing other countries in food production and exports, and the intensified use of land for agriculture have stimulated additional Amazonian deforestation. Deforestation in tropical regions is known to have large effects on biodiversity, ecosystem functioning and soil fertility. However, despite the increased appreciation of belowground microbial diversity, impacts of deforestation on the microbe networks within the soil ecosystem are largely unknown. The microbial networks reflect the complex biotic and abiotic associations as basis of soil ecosystem functioning. Here we show significant changes in bacterial taxonomic and functional networks already 2-4 months after forest clearing and burning in Brazilian Amazon primary forest and adjacent deforested sites. We found less complex taxonomy-based network in deforested soils as compared to forest soils whereas the bacterial functional network showed an opposite change. The results demonstrate that changes in the microenvironment from soils due deforestation quickly leads to simplification of the association among different bacterial taxonomic groups and in order to adapt to this condition to enhancement of function-based associations, indicating a higher degree of risk spreading for the maintenance of soil functioning. Network characterization of combinatorial fitness soil biological and chemical properties provides an approach to examine the impact of stress conditions on soil-borne bacterial communities and their functioning within the complexity of the soil ecosystem.

P44
Effect of saline irrigation water on the diversity of indigenous microbial communities in soils from a semi-arid ecosystem

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Soil salinization by irrigation water is a major threat to a sustainable agricultural production in arid and semi-arid regions. Salt accumulation has adverse effects on soil fertility and consequently on plant yields, but it may also damage soils by loosing functionally important members of the soil microbial community. The objective of this study was to characterize the diversity of bacterial communities in a pristine shrubland soil and their changes in response to increasing levels of salt introduced by irrigation water.

Shrubland soil collected near La Paz, Baja California, Mexico, was incubated in soil microcosms with or without wheat straw as a nutrient source . During this time it was irrigated weekly with non-saline (0 dS m^{-1}), low saline (2 dS m^{-1}) or high saline (11 dS m^{-1}) water, respectively. After 11 weeks, and sampling of an aliquot ("bulk soils"), germinated wheat plants were transferred into these soils . After another 7 weeks, the rhizosphere soils from these wheat plants were sampled.

In the high saline treatment wheat plants were not able to grow, whereas the high salinity treatment with the additional wheat straw allowed wheat growth, which however was negatively affected compared to the non-saline and low saline treatments. The abundance of Bacteria, Archaea and Fungi was determined by quantitative real-time PCR of small subunit rRNA gene copy numbers, amplified from directly extracted soil DNA: Copy numbers ranged from 10^9-10^{10} g^{-1} soil for Bacteria, 10^7-10^8 g^{-1} soil for Archaea, and 10^6-10^9 g^{-1}soil for Fungi, respectively. The addition of wheat straw had a strong effect on the abundance and diversity of microbial communities. Bacterial profiling by T-RFLP analysis of partial 16S rRNA genes revealed significant differences between the three salinity treatments in the rhizosphere soil; in the bulk soil differences became only apparent when wheat straw was added. The structural diversity of the bacterial and archaeal communities is currently further assessed by Illumina MiSeq sequencing of the rRNA-gene PCR amplicons. This should allow us to identify the Bacteria and Archaea which are mostly affected salinity and those which gain advantages under such extreme environmental conditions.

P45
Importance of land use change for microbial diversity in European soils

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In Europe, 25% of its land area was subjected to land use changes during the last 100 years. Land use changes affect the balance of the soils' input and output of organic material and thereby lead to altered quantities and qualities of soil organic carbon (SOC). The objective of this project is to analyze how important land use changes are for soil microbial diversity and microbial functions. Here we report on effects on soil microbial diversity.

A total of 30 paired sites in Europe were sampled, covering the major European land use change types "cropland to grassland", "grassland to cropland", "cropland to forest", and "grassland to forest". Changes in quality and quantity of SOC were assessed together with physicochemical soil parameters. Population sizes of the three domains *Bacteria*, *Archaea,* and *Fungi* were determined by quantitative PCR (qPCR) and the diversity of soil bacteria was preliminary assessed with TRFLP and joined with the above mentioned parameters in multivariate statistical analyses. These data are currently complemented with pyrosequencing of amplicon libraries obtained from 16S rRNA genes.

In a first step, soils from all sites were compared independent of the land use change: qPCR and TRFLP data revealed that the population sizes of all three domains as well as the diversity of soil bacteria were significantly affected by almost all environmental parameters e.g. the vegetation and pH. However, while population sizes depended on quality and quantity of SOC, the diversity of soil bacteria responded only to the quality.

As the next step, the impact of land use change was assessed to identify its drivers: All deviations in the environment were tested for the presence of correlations to the respective changes in population sizes or to changes in the diversity of soil bacteria. Changes in soil nitrogen and in pH were identified to explain changes on population sizes of the three domains and on bacterial diversity. However, changes in SOC quality and quantity affected the population sizes of *Bacteria*, *Archaea* and *Fungi* while changes in the diversity of soil bacteria only depended on SOC quality but not quantity. The results from high-throughput sequencing are now expected to identify the bacterial groups which respond to specific factors altered by the land use change.

P46

Exploring the prokaryotic insertion sequence abundance and diversity of Brazilian sugar-cane cultivated soils

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Introduction

The Insertion Sequences (ISs) are the smallest and simplest autonomous prokaryotic mobile genetic elements (MGEs). These MGEs play an important role in evolution, contributing massively to the lateral gene transfer. Indeed, the transposase enzyme that catalyses the IS movement are by far the most ubiquitous genes found in nature. Conversely, the sugar-cane are the most important crop of the São Paulo State (Brazil). Therefore a detailed analysis of the transposases abundance and diversity would provide an important picture of the evolutionary forces that drives the microbial communities.

Objectives

The goal of this study is to survey the IS abundance and diversity present in sugar-cane cultivate soils from Sao Paulo State (Brazil).

Materials and Methods

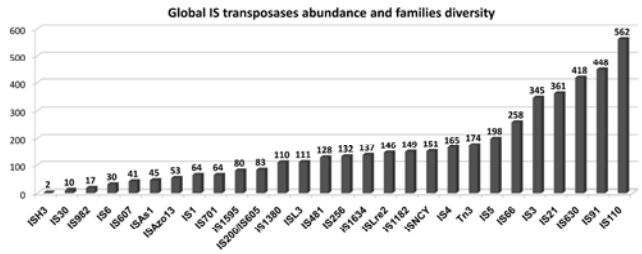
The sugar-cane cultivated soil samples were collected from the dry (SCD) and wet (SCW) brazilian seasons. The genomic libraries were constructed using the paired-end protocol (2x100bp) and sequenced using the Illumina® HiScanSQ platform. The reads were assembled using the CLC Genomics Workbench 6.5, and analyzed with MetaProdigal 2.60 software and ISsaga platform.

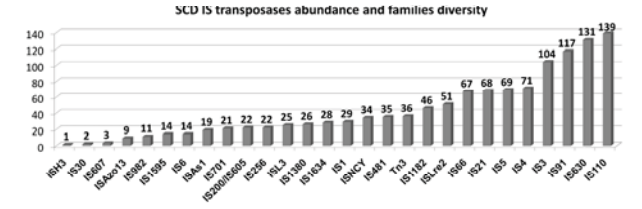
Results

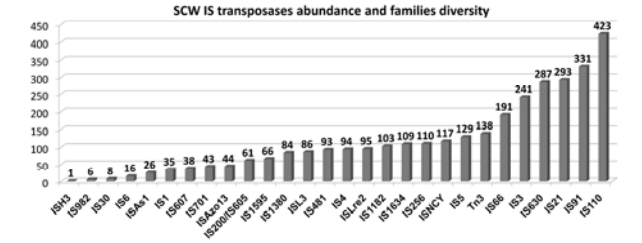
The SCD and SCW samples were assembled in 580,051 (227Mb, N50 372bp) and 1,291,242 (530Mb, N50 398bp) contigs and are composed by 148,395 and 350,051 predicted genes, respectively. At least 1,214 (SCD) and 3,268 (SCW) transposases genes representing the 28 known IS families were identified. The figure 1 summarize the main results showing the transposases abundance and diversity of each sugar-cane cultivate soil sample. In summary, for both samples, the IS110 and ISH3 families are the most prevalent and less abundant, respectively. Interestingly, 151 occurences of the non-classified family (ISNCY) were detected.

Conclusion

These results provides a considerable reservoir of unexplored and novel IS transposases on sugar-cane cultivated soil. Comparatives studies are under way and the results will be included in our future publication.







P47

Metagenomics unravels the antibiotic resistome of Indian soils

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Introduction

The development and spread of antibiotic resistance across is one of the most immense health problems in modern time. While the role of antibiotics use and abuse in resistance development has been extensively investigated, examination of the impact of environmental antibiotic pollution has been limited. We have previously shown that contamination by wastewater from antibiotic manufacturing facilities can create conditions that drive enrichment of resistance genes.

Objectives

The aim of this work was to investigate whether industrial wastewater from pharmaceutical production have an impact on the antibiotic resistome of soils in nearby villages. These soils have been irrigated with water from wells shown to have elevated levels of antibiotics compared to wells in other Indian areas.

Materials and Methods

In this work, we have utilized Illumina sequencing, which allows for broader screening of antibiotic resistance genes, as the vast

number of reads enables us to search for relatively rare types of resistance genes. Further, we have used metagenomic assembly to identify genetic elements that could be connected to antibiotic resistance.

Results
From the DNA extracted from these microbial communities, we generated more than 230 million paired-end reads, corresponding to around 16 million pairs of reads per sample. In this data, we can identify a wide range of resistance gene types. The most common resistance genes were *sul2, aph(9)-Ia*, *aac(3)-Ib* and *qepA*. However, the soils from the antibiotic-impacted area were not significantly more enriched with resistance genes or mobile elements.

Conclusion
While a range of resistance genes were detected in Indian soils, the modest contamination of antibiotics does not seem to have a substantial impact on soil microbial communities, at least not at the sequence depth of this study.

P48
Gene transfer from genetically modified plants to micro-organisms.

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Plants, whether they are genetically modified (GM) or not, live in association with numerous microorganisms including bacteria. Some bacteria are capable of integrating DNA fragments located in their nearby environment by natural transformation. In the case of GM plants, the ability of these bacteria to propagate transgenes should be evaluated. To determine whether there is a possibility of gene transfer, we tried to optimize the conditions that would induce integration and propagation of DNA in bacteria. This phenomenon essentially relies upon the existence of homologous regions between the penetrating exogenous DNA and the recipient host genomic DNA for a recombination mediated integration.
In a first series of experiments, we developed experimental conditions for maximizing the risk of gene exchange by constructing transgenic plants with the well conserved ribosomal genes as transgenes susceptible to recombine with the corresponding sequences in the genome of highly efficient naturally transformable bacteria. Transfer frequency between the plant DNA and the recipient bacteria was measured under a wide range of *in vitro* and *in planta* conditions. In a second series of *in silico*, *in vitro* and *in planta* experiments, tests involved transgenes from GM plants of pharmaceutical interest considering the bioactive potential of the transgene products.
Surprisingly, our results demonstrated that the ribosomal genes are recombination “cold” spots according to the absence of transformation events whatever the conditions tested. Similarly, the various pharmaceutical transgenes did not produce transformants with the recipient bacteria tested including when these genes were inserted in the chloroplast genome that naturally contained several homology regions with bacterial genomes.

These new series of investigations confirmed that gene transfer from transgenic plants to environmental bacteria remains very unlikely even under the conditions susceptible to increase the probability of gene transfer.

P49
Effect of amendments with excrements from CTC-treated cows on bacterial community structure in pasture soils

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Introduction
Regular treatment of farm animals with tetracyclines leads to the selection of resistant bacteria harboring various tetracycline resistance genes in animal guts, and these genes may be spread to the soil via animal feces. The possibility of transfer and persistence of gut-borne bacteria in grassland soils with different management history was tested in a microcosm experiment.

Objectives
We aimed to show (i) which bacteria are present in excrements of cows that received chlortetracycline (CTC) as a prophylaxis, (ii) how excrement addition affects the composition of bacterial communities in grassland soils, and (iii) which bacteria can persist in pasture soils amended with cow feces for at least 28 days.

Materials and Methods
Soils of pristine meadow (M), common pasture (P), and winter pasture (W) were sampled at the locality of Borová farm (South Bohemia, Czech Republic). Mixture of excrements from 3 cows was used for soil amendments, either alone or in addition with 0.2 mg/kg CTC. Bacterial community composition was assessed by 454-pyrosequencing of 16S rRNA genes at 0 and 28 days of incubation. To test differences between treatments db-RDA based on Bray-Curtis distance matrices obtained from bacterial community compositions was performed.

Results
Cow feces were dominated by anaerobic bacteria affiliated to Clostridia. M soil was dominated by Acidobacteria and Alphaproteobacteria, P soil was dominated by Acidobacteria and Betaproteobacteria, and W soil was enriched by Actinobacteria, Chloroflexi and Firmicutes. Multivariate analysis indicated a significant impact of feces amendments to each soil on bacterial community composition, while addition of CTC did not have any impact. Soils with feces amendments were enriched with Clostridia (up to 20%) and soil bacterial community structure was partially restored after 28 days of incubation.

Conclusion
Bacterial community composition of pasture soils were significantly affected by cow feces amendment and indicated that a substantial part of fecal microflora may survive in soil for at least one month.

This study was supported by Czech Science Foundation (P504/10/2077) and by Ministry of Education, Youth and Sports of the Czech Republic (COST-CZ LD13046).

P50
Broad host range vectors for expression of proteins with (Twin-)*Strep*-tag, HIS-tag and engineered, export optimized Yellow Fluorescent Protein

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In current protein research, a limitation is the production of active, recombinant proteins to experimentally assess their function. Many analyses require a controllable expression of affinity- and/or fluorescence tagged ORFs of interest in their native cellular background. A homologous and/or periplasmic expression can mostly not be realized due to a lack of suitable tools. Instead, experiments are generally limited to the heterologous production in one of the few well established expression strains.

Here, we introduce a series of new RK2 based broad host range expression plasmids for inducible production of affinity- and fluorescence tagged proteins in the cytoplasm and periplasm of a wide range of Gram negative hosts which are designed to match and extend the modular Standard European Vector Architecture. Periplasmic export variants enable production of affinity tagged proteins and generation of fusion proteins with a novel engineered *Aequorea*-based yellow fluorescent reporter protein variant with activity in the periplasm of the tested Gram-negative soil bacterium *Pseudomonas putida* KT2440 and *Escherichia coli* K12 for production or (co-)localization studies.

The new tools facilitate experimental verification of hypothetical proteins mined from soil-metagenomes and protein production yield assessment in different expression hosts possibly including new soil isolates.

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P51
Plasmid pool in pesticide-contaminated 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. This is especially important in agricultural soils, which are often treated with toxic chemical compounds, like pesticides. Soils contaminated with pesticides are often enriched in bacterial or fungi species capable to degrade deadly compounds. Moreover genes located on mobile elements are known to play important role in resistance of microorganisms to chemical pollution.

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

10 soil samples varying in the pollution level were characterized both chemically and physically, including extensive analysis of pesticides contamination. Metagenomic DNA was isolated according to Zhou et al. (2006), with modifications, sequenced using Illumina technology, then assembled with Velvet software and annotated with self developed pipeline. Incompatibility plasmid groups were resolved via standard PCR replicon typing. Presence of plasmid-originated sequences in shotgun sequences was confirmed by blast against NCBI plasmids database.

PCR replicon typing indicated that plasmids belonging to IncP-1 and IncP-9 groups are present in most of the analysed samples, whereas IncP-7 group is restricted to highly polluted soils. Analysis of contigs originated from metagenomic assembly revealed presence of numerous sequences with high similarity to known IncP-7 plasmids like: pCAR, pWW53, pDK1 and several others. Moreover sequencing data revealed DNA fragments similar to plasmids from IncP-1 and IncP-9 groups. Most of identified plasmids originate from Pseudomonas sp. family which is consistent with 16S rRNA-based biodiversity analyses.

Described analyses suggest that IncP-7-type plasmids might be important carriers of genes responsible for pesticide degradation in polluted soils.

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P52
Quantification of plasmids in soils stressed by different conditions

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Comparative whole-genome analyses have demonstrated that horizontal gene transfer provides a significant contribution to prokaryotic genomes. Some of the mobile genetic elements present in the soil communal pool of genetic information are conjugative plasmids. They participate in genome evolution and rapid adaptation to changing local environmental conditions by transferring genetic information by conjugation. The transfer of these plasmids depends on specific microbial and environmental characteristics and the ecological factors that affect the host metabolism also influence plasmid transfer as well. In soil habitats,

nutrient availability, clay/organic matter content and water availability (as examples) are believed to influence plasmid transfer by affecting the bacterial density and metabolic activity. In this study, the potential link between plasmid content and environmental conditions as a predictor of adaptability was studied. We used primers already reported in literature to quantify by qPCR for IncQ, IncW, IncN, IncP-1, IncP-7 and IncP-9 plasmids. We tested the primers in soils with different physicochemical parameters. These soils were from a forest soil from Prague, an agricultural from Munich, a sandy soil from Denmark, and an experimental field from England. We also use a highly copper contaminated soil from Hygum Denmark and non-contaminated soil from the same site to test the effect of a long term exposure to copper on the plasmid content. In addition, grassland soil was subjected to different chemical stresses in microcosms in order to test the effect of these stresses on the plasmid pool. Our results show a difference in plasmid type and content between the different soils samples and treatments. This supports the use of the primers as indicators of soil microbial community adaptability to environmental perturbations.

P53

Evaluating the mobilome of environmental samples through metagenomics

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A mobile genetic element (MGE) is defined as any discrete segment of DNA that can move within or between genomes (Siefert, 2009). Research has confirmed the role of MGEs in bacterial adaptation at the individual level however, it is important that more effort be directed towards understanding MGEs at a community level (ie. the mobilome). We are striving to understand the degree of MGE diversity in rivers with varying sources of anthropogenic stresses and are evaluating the currently available bioinformatics tools including Metavelvet (Namiki *et al.*, 2012) for characterizing mobilomes in environmental samples.

Materials and Methods

Sand filled samplers were deployed in 6 different rivers in each of the 2011 and 2012 field seasons. After three months DNA was extracted and initial comparisons were performed through DGGE and qPCR of individual MGEs. Amplicon based 16S sequencing (Roche454) was used for phylogenetic analysis using MacQIIME (Caporaso, 2010). In order to determine the minimum necessary coverage for contig assembly, Metavelvet was evaluated using both real and simulated short-read (~100 bp) metagenomic data ranging from 2 million to approximately 100 million reads. Data obtained were compared to data generated by MG-RAST (Meyer *et al.*, 2008) without assembly.

Results

DGGE and 16S analysis revealed that within sampler variations were large when the community was diverse, although sampler populations with less diversity showed greater consistency. Between river differences were not generally found to be greater than between year differences in the same river. Increased abundance of individual MGEs including increased class 1 integrons and IS1071 were detected through qPCR. Assembly of large contigs using Metavelvet required significantly greater coverage values than anticipated.

Conclusion

The suitability of this type of analysis will depend greatly on the diversity of the sample to be sequenced, and the depth of sequence coverage. As sequencing costs continue to decrease, it will become feasible to assemble metagenomic data into contigs and further our understanding of community gene content.

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P54

Amplicon sequencing and resistance gene pool of bacterial communities from wastewater irrigation fields in the Mezquital Valley, Mexico

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Introduction

Wastewater (WW) reuse for crop irrigation is widely practiced in agriculture to alleviate water shortages. The Mezquital Valley (80 km north of Mexico City) is the world´s largest WW irrigation field. WW-irrigated soils are a potential reservoir for multi resistant organisms, which might pose risks for field workers and consumers of the agricultural products.

Objectives

To study the composition of the bacterial soil communities and the resistance gene pool in ww irrigated soils in comparison with rain-fed soils to assess the impact of WW-irrigation on the bacterial communities.

Materials and Methods

Sampling was done twice to study differences between dry and rainy season. Pyrosequencing of amplicons of the V2-V3 region of the bacterial 16S rRNA genes from different soils (non-irrigated, irrigated for 24 and 100 years from the rainy season and non-irrigated and irrigated for 100 years from the dry season) was performed. We sequenced 192 bacterial isolates from a rain-fed soil and a soil irrigated for 100 years and determined the resistance towards twelve antibiotics by antimicrobial susceptibility test. All isolates were tested for the presence of *sul* and *qnr* genes by PCR.

Results
We observed a community shift towards an increase of *Proteobacteria* after WW irrigation. 11% more *Proteobacteria* (two fold more γ *Proteobacteria*, e.g., *Pseudomonas, Acinetobacter*) were detected in the WW-irrigated soils from the rainy season, indicating a larger prevalence of potential pathogens in WW-impacted soils. In the dry season 30 % more *Proteobacteria* were detected in WW-irrigated soil. Most isolates from WW-irrigated soil belonged to the *Proteobacteria* (e.g., *Stenotrophomonas, Pseudomonas*) and Firmicutes (*Bacillus*). All isolates from rain-fed soil were affiliated to Firmicutes (*Bacillus*). The isolates showed up to six resistances, resistance to sulfamethoxazole (22.9 %), followed by resistance to oxacillin (9.4 %), being the most abundant in WW-irrigated soils. However, no *sul* and *qnr* genes were detected in the isolates.

Conclusion
We observed an increase of potentially pathogenic bacteria and isolated more multi-resistant bacteria from WW-irrigation fields which might cause health risks for people in the Mezquital Valley.

P55
Widespread dissemination of class 1 integron components in soils and related ecosystems as revealed by cultivation-independent analysis

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Introduction
Class 1 integrons are able to acquire, exchange, and express resistance genes embedded within gene cassettes. Besides the clinical setting they were recently reported in environmental habitats and often located on plasmids and transposons, facilitating their transfer and spread within bacterial communities.

Objectives
In this study we aimed to provide insights into the occurrence and diversity of genes typically associated with the clinically relevant class 1 integrons in different environments with or without human impact and their association with IncP-1 plasmids.

Materials and Methods
Total community DNA from different soils, biogas plant digestates, manure, and on-farm biopurification systems was extracted and screened by PCR with subsequent Southern blot hybridization for the presence of the class 1 integrase gene *intI1* as well as *qacE* and *qacEΔ1* resistance genes. Furthermore, the abundances of *intI1, qacE/qacEΔ1,* and *sul1* genes were quantified relative to 16S rRNA gene abundance by quantitative real time PCR in three different soils and in the rhizosphere of lettuce. The results were compared to the abundance of *korB* genes specific for IncP-1 plasmids to assess their potential involvement in the spread of class 1 integrons. Additionally, 28 IncP-1ε plasmids carrying class 1 integrons, which were recently captured exogenously from piggery manure and soils treated with manure were further tested for the presence of *qacE/qacEΔ1* genes.

Results and Conclusion
The present study expands the environmental settings in which class 1 integrons were detected and thus are in line with the previously reported dissemination of these genes in natural environments and environments with anthropogenic impact. The class 1 integrons were mainly related to the presence of *qacEΔ1* genes and IncP-1 plasmids likely contributed to the spread of this integron class in the analyzed soils. However, the IncP-1ε subgroup, previously identified as important vector of antibiotic resistance genes in agricultural systems, was only found in low abundance suggesting a minor contribution to the presence of class 1 integrons in the investigated arable soils.

P56
Biodegradation of polyester polyurethane buried under compost at different temperatures

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Plastics form an essential role in the modern world due to their low cost, insulating properties, light weight and durability. However, accumulation of plastic waste in the environment is responsible for unique and long lasting effects and managing waste plastics is increasingly expensive. Polyurethanes (PU's) are a high volume plastic that make up ca. 7% of the total plastic production in Europe with demand increasing every year. PU's are heteroplymers and polyester PU's in particular have been extensively reported as susceptible to biodegradation in the environment, particularly by the fungi. In this study, we investigated the impact of composting on PU's as composting is a microbially rich process that is increasingly being used for the processing of green and food waste as an economically viable alternative to landfills. PU coupons were incubated for 12 weeks in fresh compost from the maturation stage at 25°, 45° and 50°C to emulate the mesophilic, maturation and thermophilic stages of the composting process. Incubation at all temperatures caused significant physical deterioration of the polyester PU coupons at all temperatures and was associated with extensive fungal colonisation and loss in tensile strength. By contrast, no loss in tensile strength was detected in polyether PU coupons over the 12 week incubation period. A number of fungal species were isolated from the surface of polyester PU coupons at 25°C while only *Aspergillus fumigatus* and *Thermomyces lanuginosus* was recovered at 45° and 50°C respectively. TRFLP and pyrosequencing of the fungal communities on the PU surface and in the surrounding compost revealed that the population on the surface of PU was different from the community observed in compost suggesting enrichment and selection on the surface of PU coupons. The most dominant fungi identified from surface of PU coupons by pyrosequencing were *Fusarium solani* and *Bionectria ochroleuca* at 25°C, while at both 45° and 50°C *Candida ethanolica* and an unidentified fungal clone were dominant. This preliminary study suggests that the composting process has the potential to biodegrade PU waste if optimised further in the future.

P57
Selection and metataxonomic characterization of soil microbial communities involved on wheat straw bioconversion

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In the last decade, a high industrial demand for efficient multispecies-consortia that allow the bioconversion of different

lignocellulosic substrates has emerged. The biodegradation of lignocellulosic biomass and subsequent production of green chemistry/renewable fuels through complex communities has been proposed as a very promising biotechnological approach, which, however, is in continuous need of improvement. Hence, novel genes/enzymes and bacterial-fungal players are deemed to be very important for use in consolidated bioprocessing (CBP). In order to characterize the diversity and composition of two novel multi-species microbial consortia involved in the bioconversion of lignocellulose, we reported a metataxonomic analysis of two aerobic wheat straw microcosms cultures constructed by dilution-to-stimulation approach. Forest soil as microbial source and three sequential-batches were evaluated by quantitative PCR and amplicon-pyrosequencing. The abundance of bacterial communities was approximately 2.61 (± 0.59) log copies per ml higher than the fungal one along the transfers. A total of 18,200 and 6,600 trimmed-rarified sequences of 16S rRNA and ITS1, respectively, were analyzed. The Faith's phylogenetic diversity for bacterial and fungal communities became markedly reduced from the soil inoculum and throughout the sequential-batches. Both systems appeared to indeed incite a reshaping of the bacterial communities, with reductions in richness and increases in prevalence of particular members of the *Enterobacteriales*, *Pseudomonadales*, *Flavobacteriales* and *Sphingobacteriales*. Among the fungal players with high biotechnological relevance, co-existing with bacteria ones, we detected member of the genera *Coniochaeta*, *Acremonium*, *Aureobasidium*, *Penicillium*, *Cryptococcus* and *Trichosporon*. The community composition of lignocellulose-responsive bacteria and fungi was strongly influenced by substrate treatment, suggesting the presence of furanic compounds, lignin monomers or cello-oligosaccharides in the treated wheat straw culture. The two novel consortia may constitute starting points to biotechnological applications, construction of metagenomic libraries, and might enhance our understanding on the microbial lignocellulose bioconversion in engineered or natural soil environments.

P58

The role of mobile genetic elements in pesticide biodegradation in on- farm biopurification systems

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Introduction
Biopurification systems (BPSs) on farms remove high concentrations of pesticides from wastewater via sorption and bacterial degradation. The selective pressure drives the evolution of novel enzymatic activities and metabolic pathways in bacterial communities to use such organic xenobiotics as sole source of energy and carbon or as a nutrient source. Genes involved in the degradation of organic xenobiotics are often located on mobile genetic elements (MGEs). Broad host range IncP-1 plasmids and the composite IS*1071* transposons are among the most prevalent MGEs that carry xenobiotic catabolic gene clusters. The catabolic functions associated with these mobile elements however are still to be discovered.

Objectives
The aim of this study is to discover novel amidases and further elucidate pesticide degradation pathways in BPS microbial communities.

Material and Methods
The pesticide at the focus of this study is the phenylurea herbicide linuron. BPS material was collected from different on-farm BPS systems as well as BPS microcosms to which linuron was applied. Accessory genes of IS*1071* transposons or IncP-1 plasmids were amplified by Long range (LR) PCR and sequenced. Amplicons of 10-15 kb were cloned and the resulting clones were screened for linuron degradation. In addition, the putative amidases from the gene clusters were cloned individually. The clones expressing the amidases were tested for their ability to use various xenobiotics as their sole nitrogen source.

Results
High prevalence of IncP-1 plasmids and *IS1071* elements were observed in pesticide treated microcosm and in on-farm BPS. Genes previously reported to facilitate the degradation of linuron were retrieved from both the microcosm and the operational BPS.

Five putative amidase genes were retrieved from the amplicon sequences one of which was present in both the operational BPS and the microcosm. These genes had % identities to their nearest neighbors varying between 79 and 37, suggesting that they may encode for novel amidases.

Conclusion
Our data show the extensive catabolic potential of microbiota in a BPS at the genetic level and suggest that the mobilome is an important mediator in shaping this genetic content. Future goals focus on the biological verification of the bioinformatics data derived from the mobilome.

P59

Methane production from microalgae at high and at normal pH - Metagenomic and metatranscriptomic analyses of Alkaline and mesophilic biogas reactors

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Biogas is a renewable energy source already produced at large scale, however, this usually requires the use of energy crops as substrate, which compete with food and feed plants for arable land.

Microalgae can be used as alternative substrate, not requiring the use of agricultural land. To prevent the cost intensive process of air or CO_2 sparging, microalgae can be cultured at high pH (9.5 to 10). The increased solubility of CO_2 in high pH media would also be advantageous for the biogas production from these algae, since a biogas reactor operating at high pH would act as CO_2 scrubber, yielding biogas richer in methane.

Microbial communities in conventional biogas plants are, however, sensitive to pH values above 8.5. Thus, for high pH biogas reactors, an adapted microbial community needs to be established.

Soda lakes are alkaline habitats in which methanogenesis occurs at pH values between 9 and 12. Sediment from these habitats can be stored over longer periods while still retaining it's methanogenic activity[1].

We present the metagenomic and metatranscriptomic studies of two lab-scale biogas reactors, using the microalgae Spirulina as substrate. One reactor was inoculated with sediment from soda lakes, operating at pH 10 and 2M Na^+, the other operating at mesophilic conditions as a control.

Whole metagenome DNA shotgun sequences were assembled and binned, and the results compared to 16S amplikon metagenomic sequencing. Taxonomic analyses showed that there are similarities regarding the general composition of the metagenomes from the two reactors, but distinct differences at lower taxonomic levels, revealing the adaptation of each community to either alkaline or mesophilic conditions. Total mRNA sequencing showed that despite of their low abundance, methanogenic Archaea are an active population of the biogas reactor community.

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P60

Potential of petroleum hydrocarbon biodegradation from Trindade Island coastal soils, Brazil.

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The Trindade is an oceanic island of volcanic origin, 1.200,00 km east of Vitória, the coastal capital of the state of Espírito Santo, South Eastern, Brazil. Trindade Island is the closest oceanic island from the main brazilian petroleum offshore exploration area. It has been described a high level of fauna and flora endemism at Trindade Island. The geografic isolation and the particular type of soil present there, supply the conditions to new species emmergence. The hydrocarbon toxicity, mutagenicity and carcinogenicity offers danger to the sea life close to the exploration area. The objective of this work was to evaluate the differences in soil microbial community under the contamination with crude oil at this particular environment. The samples were taken in triplicates from beach sand and soil under native vegetation in two different areas. One beach is turned to Africa´s coast and the other one is turned to Brazilian coast. The soil collected were cooled and transported to the Laboratory of Environmental Biotechnology and Biodiversity, Federal University of Viçosa. Samples were assembled in microcosms (350 g), and treated with crude oil in a concentration of 30 g kg^{-1}, and further incubated in a greenhouse for 30 days. We evaluated the respiratory rate, the carbon of microbial biomass, the growth of bacterial on TSA and BH mineral medium with petroleum as the only source of carbon and the petroleum hydrocarbon degradation during the incubation time. The genetic material was extracted to evaluate the microbial diversity by PCR multiplex T-RFLP analysis, as well as RNA samples for future works on the microbial gene expression. As results we had the increase of respiratory rate, the increase of metabolic quotient and the increase on microbial biomass due the contamination. We interpreted all those increases as an answer to the stress imposed by the petroleum as well as the changes in biodiversity. The next steps will be to evaluate changes in functinal genes during the contamination by "shotgun" DNA sequencing.

P61

Metagenomic/transcriptomic analysis of the cathode associated community of a bioelectrical system

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Introduction

Extracellular electron donors can be an important component in some soil and sediment environments. An autotrophic, aerobic microbial consortium was enriched from a microbial fuel cell originally inoculated with sediment and seawater, using a graphite cathode as an electron donor.

Objectives

This microbial consortium is being investigated using a systems biology approach to identify the molecular mechanisms the constituent organisms use for electron transport, carbon fixation, and biosynthesis. The end goal of this project is to develop a platform that will enable biotechnological exploitation of microbial cathode communities.

Materials and Methods

The microbial consortium was grown on graphite or carbon cloth electrodes poised at 310 mV versus a standard hydrogen electrode. Metagenomic libraries were sequenced using an Illumina HiSeq 1000. Metatranscriptomic libraries and variable regions of the 16S rRNA gene were sequenced with an Illumina MiSeq, and the expression levels of genes identified in the metagenomes were quantified. Several organisms were isolated or enriched from the consortium, and those isolates are being sequenced. Fluorescence activated cell sorting (FACS) based upon fluorescence *in situ* hybridization of 16S rRNA is under way in an attempt to obtain highly enriched populations of single ribotypes for genomic sequencing.

Results

Multiple lines of evidence including classification based upon 16S rRNA sequencing, genomic assignments of genes identified in the metagenome, and cultured representative indicate that the consortium is dominated by Gamma- and Alphaproteobacteria. This was consistent among the eight biological replicates. Two contigs contained the CO_2 fixation genes *rbcLS*, both with some affiliation to sulfide and thiosulfate oxidizing bacteria. Metatranscriptomic data is currently being analyzed to identify potentially important pathways for energy and carbon distribution among this community.

Conclusions

This microbial community is capable of fixing CO_2 during growth on an electrode. Further analysis of metagenomic and metatranscriptomic data, using sequencing data from isolates and FACS enrichments will allow for a more detailed characterization of this community which will allow for further development of bioelectrical synthesis.

P62
Group-specific pyrosequencing to identify low abundant Clostridia in a biogas plant

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Bacteria of the genus *Clostridium* are common members of the consortium of microorganisms that produces biogas. Since the genus *Clostridium* also harbors some highly pathogenic members in its phylogenetic cluster I, e.g., *Clostridium botulinum*, there has been some concern of an unintended growth of such pathogens during the fermentation process. However, the knowledge on the abundance and diversity of Clostridia in biogas plants is still very limited and was thus address in this study. Populations of *Bacteria*, *Archaea* and Clostridia were quantified by qPCR in samples from an anaerobic digester, the respective fermentation residue storage tank and in the substrates used for biogas production. *Clostridium* cluster XIVa was selected for quantification to indicate fecal Clostridia, while cluster I includes potentially pathogenic members. These data were complemented by pyrosequencing of amplicons obtained by a group specific approach that selectively targeted members of *Clostridium* cluster I.

The population sizes of Clostridia varied between the substrates analyzed in this study, i.e., cattle manure, ensiled grass or ensiled maize. The 16S rRNA gene copy numbers of *Clostridium* cluster XIVa 16S rDNA gene copies represented between 2 and 10% of the total bacterial 16S rDNA gene copy numbers, while the members of *Clostridium* cluster I represented between 1 and 5%. In the anaerobic digester the content of both clusters was affected by the kind of substrate mixture added: *Clostridium* cluster XIVa was present in the range from 3 to 6% while cluster I was between 2 and 5%. These contents were reduced in the storage tank receiving the organic residues from the digesters.

Pyrosequencing revealed 260,000 rDNA gene sequences that could be assigned to *Clostridium* cluster I. Preliminary analyses of the sequencing results indicate 16S rDNA gene sequences related to *C. botulinum* at ≥ 97% similarity in 4 of 12 samples. The highest incidence represented 0.0047% *C. botulinum* affiliated sequences of the total bacterial 16S rDNA genes. The detection of these rare events stresses the importance of group specific high-throughput sequencing approaches to support a microbiological risk assessment of biogas production processes.

P64
Edaphic, environmental and spatial drivers of microbial communities of Australia soils

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Introduction
Soil microbial communities mediate many important ecosystem processes and the drivers of microbial community structure are of great interest. The Australian continent has long been separated from other continents, has a large area that spans across distinct climatic zones and contains many diverse habitat types. Climate projections for many Australian regions suggest a warmer and drier future with greater extremes relative to current conditions. An understanding of the determinants of microbial community structure may allow better understanding of potential climate induced changes to these communities and the ecosystem services they provide.

Objectives
An understanding of the relative contributions of edaphic, climate and spatial factors to determining microbial community structure.

Methods
Soils were sampled from 100 sites across Australia as part of the Biomes of Australian Soil Environments (BASE) project. From these soils 16S rRNA and ITS tag sequences were 454 pyrosequenced from surface (0-10cm) and subsurface (20-30cm) depths (200 samples). Soil edaphic (e.g., pH, C, N, P, EC, moisture, etc.), climate (average temp., rain, aspect, overlying vegetation composition) and spatial (geographic position) variables were also obtained for each sample. Soil variables were correlated with bacterial and fungal OTU's to determine their relative contribution explaining community structure. A model was constructed to predict microbial community structure from contextual variables.

Results
Bacterial and fungal soil communities exhibited significant variation that was strongly associated with edaphic variables. Climate and geographic distance also explained variation in community structures, although to a lesser extent. We were able to predict dominant community members under certain soil conditions within defined geographic regions.

Conclusions
Despite long isolation from other world regions and the presence of many distinct habitat types, the microbiota of Australia soils exhibited similar biogeographic patterns to those reported elsewhere. Edaphic factors explained the majority of community variation, followed by climate and geographic space. Our model predicts that as soils become drier their communities may converge to be more similar to the more arid communities we sampled. Future work will further test this model and attempt to incorporate temporal variability, which is expected to increase with climate change.

P65
Soil Bacterial Biogeography in the Western Swiss Alps

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The possible effects of climate change on biodiversity is of major concern for scientists, policy-makers, and laypeople. The wide elevational gradients and topographical heterogeneity in the Alps present a unique opportunity to study the effects of climate and land-use changes on this biodiversity. Indeed, the mean annual temperatures in the Swiss Alps have increased by 0.57ºC per decade, while the northern hemisphere has increased by 0.25ºC per decade. Over the last decade, an ongoing project at the University of Lausanne has extensively investigated plant-plant and plant-insect interactions within a 700 km2 area of the Western Swiss Alps, and used these data to model and predict niche-based migration patterns in a possible future with a changed climate.

However, despite the exhaustive scientific resources available for macroorganisms and abiotic processes, there is currently no systematic data available on the microbial diversity associated with the study sites. In order to fill this knowledge gap and to allow us to study the alpine biodiversity from a more holistic perspective, we have begun to investigate the bacterial community diversity in the alpine top-soils across an elevational gradient of 500-3000 m at the same sites where plant and insect data have been previously collected. We have optimized a workflow using a tripartite barcoding system and paired-end HiSeq Illumina sequencing of the V5 hypervariable region of the 16S rRNA gene, in order to obtain deep sequencing coverage across multiple soil metagenomic DNA samples. This presentation will show first results of the bacterial community analysis in the alpine ecosystem and interpretations of alpha and beta diversity.

P67
Expanding the soil fungal diversity through barcoding and illumina paired-end of the ITS1 region

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Next-generation sequencing of conserved phylogenetic markers have improved estimations of fungal diversity and have opened promising opportunities for assessing their ecological roles in the environment, such as in soil dynamics or for interactions with plants. In the last few years, the fungal internal transcribed spacer (ITS) region has become established as a molecular marker for diversity studies. However, because of the variable length of the ITS in fungi, and because of biases in covering all important fungal groups by the current choice of ITS primers, it is challenging to correctly interpret fungal diversity from ITS sequence reads. In this work, we revisited the design of primers for the fungal ITS1 region and proposed an approach using paired-end reads. Based on a large ITS1 alignment we designed 20 new barcoded primers, that enable amplification of the ITS region from metagenomic DNA and subsequent Illumina paired-end sequencing.

We studied the quality of the developed primers and the impact of barcoding the primers on ITS amplified from five soil samples and repetitions, purified from the Swiss Western Alps. Overall, seven million 160 bp concatenated reads were obtained, which were clustered and assigned to fungal taxonomic groups using a Qiime 1.7 and a curated database. The alpha and beta diversity analysis showed non significant variation among replicates at the same site, but significant variation between Alpine sites. We discuss the clustering, taxonomic assignment and their implications in interpreting fungal diversity in the environment.

P68
Soil hydrophobicity and greenhouse gas flux dynamics: An integrated approach

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Introduction
Soils represent the largest store of the world's terrestrial carbon (C). It can become a net source of carbon dioxide (CO_2) and methane (CH_4), if C mineralization exceeds its accumulation in soil organic matter and hence increase total greenhouse gas emissions. Methanogenesis occurs in anoxic environmental habitats i.e. water-logged soils, while CO_2 is a by-product of aerobic metabolism. Key genes for methanogenesis and methanotrophy are mcrA and pmoA respectively. Soil Water Repellency (SWR) is defined as the reduced infiltrative capacity of a soil toward water. A feature of many soils, SWR occurs after dry spells and has a number of implications including reduction in soil water retention and accessibility of metabolic substrate for microbes. Since SWR is intimately linked with soil moisture, it is proposed to have a significant impact on soil-atmosphere carbon fluxes including reduced soil CO_2 efflux and increased CH_4 uptake.

Objectives and Methodology
Gas sampling followed by GC-FID and LICOR analysis was utilised to quantify the in situ CH_4 and CO_2 dynamics from soils with a diverse severity of SWR at different depths and moisture contents. The effect of soil moisture content was determined under standardised laboratory conditions for intact and homogenised soil samples. Dynamic changes in microbial community composition and abundance of key genes for methanogenesis (mcrA) and methanotrophy (pmoA) under different SWR conditions are determined using next-generation sequencing approaches.

Conclusion
The investigation of CO_2 and CH_4 fluxes from water repellent soils is addressing a major research gap and aims to lead to novel strategies for managing SWR as a means to optimise C sequestration in soil.

P69
Illumina metabarcoding of a soil fungal community

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Next generation metabarcoding is becoming an indispensable tool in fungal community ecology. Here we tested Illumina metabarcoding, a method that generates shorter reads but achieves deeper sequencing than the 454 metabarcoding approaches. We found that paired-end Illumina MiSeq data cover the full ITS1 in many fungal lineages and are suitable for environmental fungal community assessment. There was substantial read loss during data cleanup (78.6%), which, however, did not impede the analyses, because of the large number of initial sequences (over 4Mio). We observed a high stochasticity in individual PCR reactions. Comparing three repeated sets of PCRs products showed that 58.5% of the total fungal operational taxonomic units (OTUs) found were not recovered by any single set of PCR reactions. Similarly, comparing three annealing temperatures showed that 63.6% of all fungal OTUs were not recovered using any single annealing temperature. These findings suggest that sampling of soil fungal communities is more exhaustive, if we combine repeated PCR products, and PCR products generated at various annealing temperatures. To analyze

the above issues we sampled 16 soil cores along a 270 cm transect in a meadow. In total we recovered 3320 fungal OTUs (based on a 95% similarity threshold). Distance decay analysis indicated that community similarity decreased slightly with geographical distance.

P70
Pre-evaluation of an old creosote polluted site for phytoremediation: spatial variation of PAH contamination, geochemical properties and microbial communities

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The design of successful bioremediation demands a thorough knowledge of the indigenous microbial populations in the polluted site. High spatial heterogeneity of pollution levels and soil microbial communities present a complication in implementing bioremediation. Although spatial variation in abundance and diversity of soil organisms has long been considered ‘‘noise’’ in microbiological studies, it has in several studies been shown that various free-living microbial populations display clear spatial patterns. The spatial patterns of microbial distribution primarily arise from their dependence on spatially variable soil geochemical parameters.

The objectives of the present study were to characterize the spatial heterogeneity of the soil microbial community at a former wood-treatment site contaminated with creosote. A grid design sampling was performed on the site and 16S rRNA pyrosequencing was used to assess the bacterial community structure.

Basal respiration assay and FDA hydrolysis assay for enzymatic activity were carried out as a measure of microbial activity. Geostatistical analysis revealed the spatial relationship between the phenotypic community composition, microbial activity and geochemical soil variables, such as PAH concentrations, soil solution pH, electric conductivity, total organic carbon (TOC), and particle aggregate distribution.

We noticed a strong correlation of the spatial distribution patterns of different phyla with the geochemical parameters. Alphaproteobacterial abundance was higher in the hotspots of PAH pollution whereas Actinobacterial abundance followed the opposite trend. A pH gradient was found on the site which was shown to define the spatial distribution of Acidobacteria. The microbial activity varied spatially with the distribution of pollutants and TOC. The use of pyrosequencing and soil microbial activity combined with chemical/physical data revealed highly relevant information for implementing bioremediation on the site.

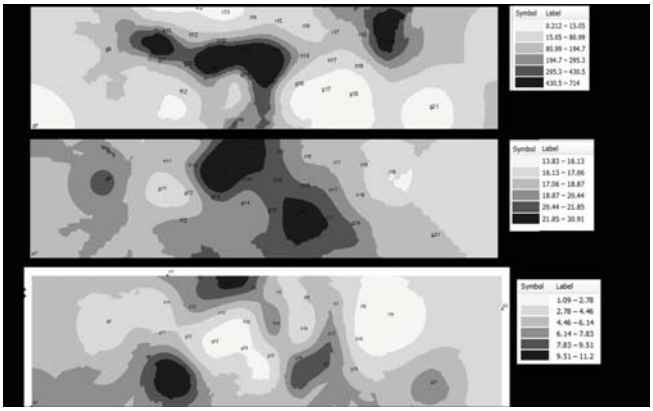

Fig. 1. Krigged maps of the distribution of A) PAH concentration, B) Alphaproteobacterial relative abundance and C) Actinobacterial relative abundance in the polluted site. Letters represent the sampling grids; 5 subsamples were collected from each grid.

P71
Microbial Ecology of Exotic Plant Invasions

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This collaborative study between MPG Ranch and the Earth Microbiome Project (EMP) focuses on changes in rangeland soil ecology driven by invasion gradients of three exotic plant species (cheatgrass, knapweed, and leafy spurge). Prior work at MPG Ranch has shown that invasive forbs can increase the abundance and diversity of arbuscular mycorrhizal fungi in the underlying soil, demonstrating a coupling between aboveground and belowground processes. In order to investigate this pattern more thoroughly, samples were collected along linear transects across three invasion gradients. Replicate ‘invaded’ and ‘native’ sites were sampled across MPG Ranch. Amplicon and metagenomic sequencing were used to characterize the bacterial, archaeal, and fungal communities. Surveys of plant community composition were also carried out. Our results suggest that soil bacterial and archaeal diversity is negatively correlated with plant diversity along a grass invasion gradient, and positively correlated with plant diversity along forb invasion gradients. Invasive plants appear to alter the network structure of microbial communities relative to native prairie, and several putative indicators for transitions between native and invaded ‘states’ were identified. The results show evidence for a balance between deterministic (niche) and stochastic (neutral) processes in shaping microbial community assembly in native Montana rangeland. This balance is tipped towards niche processes along a grass invasion gradient, and towards neutral processes along invasive forb gradients. We suggest that the successional state of the system and/or resource availability is altering the dominance of deterministic vs. stochastic assembly rules. Overall, our results demonstrate a strong coupling between plant community composition and microbial community structure and function.

P72

Microbiome impact assessment of iron oxide nanoparticles used for bioremediation of hydrocarbon contaminated aquifers.

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This project aims to enhance microbial remediation of hydrocarbons in groundwater via injection of iron-oxide nanoparticles (Fe-Ox NP). Fe-Ox NP can enhance microbial iron reduction in pure culture (Bosch et al., AEM 2010) but little is known about the effect of Fe-Ox NP on natural microbial communities and their potential ecotoxicity. In this study we investigate the ecotoxicology of selected Fe-Ox NP on groundwater microbial communities and higher organisms (nematodes) important in aquifers.

To infer potential impacts of Fe-Ox NP application on overall microbiota in sediments, microcosms containing uncontaminated reference sediment were incubated with different treatments (varying concentrations of NP and contamination) over a period of 189 days. Ecotoxicological tests demonstrated increasing toxicity of NP on nematodes compared to sedimentary microbial communities. Results of community fingerprinting analyses revealed that increasing concentrations of NP in sediments caused an initial rearrangement of microbial communities, followed by a return to the original community composition, inferring a low ecotoxicity of NP *in situ*.

Furthermore, sediment and groundwater samples taken from a contaminated aquifer used for our pilot project were subjected to a depth resolved examination of the microbial communities in the sediments using pyrosequencing coupled with community fingerprinting. A homogeneous distribution and a generally high similarity of bacterial communities was found over depth. Sequence information revealed a high abundance of microbes typically found in electron-acceptor limited BTEX-degrading environments, including a dominance of *Deltaproteobacteria* and a high abundance of *Anaerolineaceae* (*Chloroflexi*). This information serves as the basis for the identification of potential community changes or rearrangements occurring after the injection of Fe-Ox NP directly into the pilot aquifer in October 2014. Overall, this is the first comprehensive lab and field study systematically addressing microbiome impacts of NP to be used in contaminated site remediation.

Reference

Bosch, J.; Heister, K.; Hofmann, T.; Meckenstock, R. U. Nanosized iron oxide colloids strongly enhance microbial iron reduction *Appl. Environ. Microbiol.* 2010, 76 (1) 184- 9.

P73

Shifts in microbial communities in response to long-term silver exposure

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An increasing tendency to use silver (Ag) as an antimicrobial agent in commercial products may increase the flow of Ag through the wastewater-biosolids-soil environmental release pathway. This may cause the selection of Ag resistant bacteria or even spreading of Ag resistance via the bacterial mobilome.

In this study we investigated the effects of Ag on the soil bacterial abundance, α and β diversity, while we also screened for Ag selected populations.

Soils from five sites were treated with Ag at multiple concentrations ranging from 50 - 400 mg/kg and samples were obtained after incubating at 20°C for 2 weeks (2W) and 9 months (9M). Soil physicochemical properties and bioavailable Ag (by DGT) were also measured. 100 soil samples were screened for copy numbers and diversity of the 16S rRNA marker gene with quantitative real-time PCR and Illumina high-throughput sequencing of PCR amplicons.

Large physicochemical and bacterial community differences were identified between soils of different sites. 16S rRNA gene counts showed a rapid decline in bacterial numbers in the Ag-treated samples with the induced reduction being maintained also after 9 months. Inversely, the 3 % sequence distance operational taxonomic unit (OTU) α-diversity showed an increase in the Ag treated samples. Distance based redundancy analysis showed that time of exposure to Ag (primarily) and the applied doses (secondarily) explained 55-78 % of the observed variance in microbial community structures within sites. With respect to dominant OTUs, an OTU from one site consistently increased from ~0.2 % in the control and 2W samples to ~3.5 % in the 9M samples. Placement of its sequences into a full gene length maximum likelihood tree resulted in saturation of slowly growing *Mycobacteria*, with the sequences residing in the immediate vicinity or within the *M. tuberculosis* and *M. avium* complexes.

Our results show that the tested Ag doses reduced soil bacterial counts and increased the measured diversity. Moreover, the initial communities contributed to the selection of potential pathogens.

P74

Microbial community structure at habitat level controls the response to antibiotics

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Introduction

The agricultural consume of antibiotics increases. Most of the applied veterinary antibiotics such as sulfadiazine (SDZ) are excreted by the medicated animal and are transported with the manure to soil. In soil, SDZ remains bacteriostatic and alters the microbial community. Antimicrobial effects on soil microbial activities, functions, resistance, structures are known. Nonetheless, the microbial communities vary among different soil habitats such as rhizosphere, earthworm burrow and macroaggregate fractions,

and it is expected that this might affect their response to the applied SDZ.

Material and Methods

A suite of laboratory and field experiments was conducted, using the same Luvisol with sampling times from 14 up to 252 days. Soil of laboratory experiments was contaminated with SDZ-spiked manure, while for the field experiment manure from medicated pigs was used. Soil fractions of rhizosphere, earthworm burrow and macroaggregate were sampled with four replicates. Samples were stored at -20°C before characterizing the soil microbial community and the SDZ effects with phospholipid fatty acid (PLFA) of microbial markers and denaturing gradient gel electrophoresis (DGGE) fingerprints of *Pseudomonas* and *ß-Proteobacteria* 16S rRNA genes. Data evaluation was done by principal component analysis (PCA), two-way ANOVA and post-hoc tests.

Results

The results show that the SDZ concentrations and microbial responses were different and particularly larger in earthworm burrows and soil macroaggregate surfaces. In rhizosphere soil, the SDZ concentration and some microbial SDZ effects were comparable to the bulk soil. Nonetheless, microbial reactions to SDZ were clearly different and even more pronounced in rhizosphere soil in laboratory at larger contamination levels. The study confirmed that microbial communities of diverse soil habitats can react differently to the application of SDZ-contaminated manure, particularly in soil of larger microbial activity and abundance. An exploration with pyrosequencing and quantitative polymerase chain reaction (qPCR) is sought to get further insides into the habitat-specific antibiotic effects on structural and functional diversity.

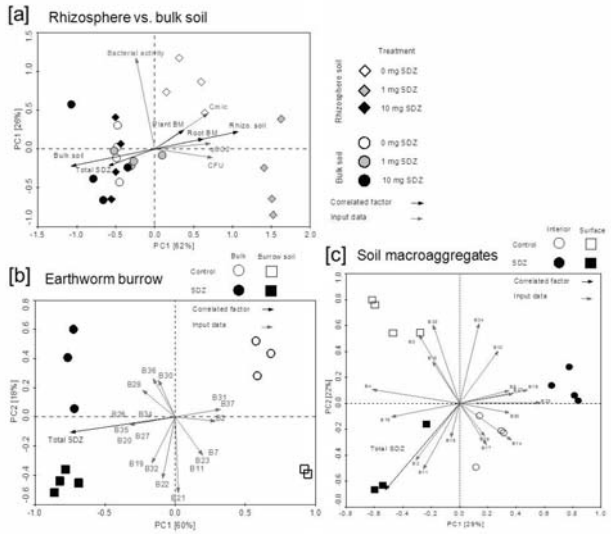

Figure. Principal component analysis (PCA) of different microbial input data of different soil habitats: [a] PCA of several standardized measures C_{mic}, qCO_2, CFU, and bacterial activity in rhizosphere/bulk soil; [b] *Pseudomonas* DGGE of earthworm burrow/bulk soil; [c] PLFA patterns of soil macroaggregate interior/surface.

P75

A temporal metagenomic survey of soil microbial community following willow planting in petroleum hydrocarbon-contaminated soil

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Soil contamination attributed to toxic pollution from industrial sites is a serious problem worldwide, with a public health impact comparable to Tuberculosis, HIV/AID or Malaria. Among the different existing depollution approaches, phytoremediation proved to be extremely cost-effective and environment-friendly, but its underlying mechanism is poorly understood.

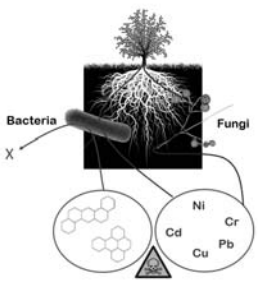
This study is aimed at understanding how the original soil microbial community surrounding plants is affected by petroleum contamination, and how it reacts to pollutants over time.

Willows were planted in crude-oil contaminated (900-1500 mg/kg C10-C50 and 60 mg/kg HAP), and non-contaminated soils in a greenhouse and the taxonomic and functional composition of microbial communities was monitored in the rhizosphere and the bulk soil at three different time points using deep Illumina sequencing of genomic DNA.

Our comparative analysis shows that the overall soil's bacterial content is moderately affected in contaminated conditions with a moderate but significant promotion of some known hydrocarbon-degrading taxa mainly belonging to β and γ-proteobacteria. However, the microbiome of contaminated soil tends to resemble the non-contaminated one after less than one year. Interestingly, we observed that contaminated soils exhibit a highly significant increase in plasmid and virus-related sequences, and that these mobile entities carry genes involved in metal-trace element resistance and petroleum hydrocarbon degradation.

Taken together, our results show that soil's microbiome possesses the intrinsic capacity to overcome petroleum contamination over a relatively short period of time. Our data confirm that known petroleum degrading taxa are implicated in this remediation process and suggest that a community of generalist bacteria could also participate in this process through the acquisition of new genes carried by plasmids and viruses. These findings open new perspectives to improve bioremediation processes.

GenoRem



P76

Comparative Metagenomics unravel adaptive evolution processes in *Dehalococcoides mccartyi*

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Introduction

Some strains of *Dehalococcoides mccartyi (Dhc)* are capable of completely detoxifying chlorinated ethenes, widespread and harmful groundwater contaminants. *Dhc* genome sequencing demonstrated the presence of multiple non-identical reductive dehalogenase (RDase) genes in strains obtained from contaminated sites and pristine environments. The development of communities comprising *Dhc* with RDase genes enabling reductive dechlorination to ethene at some sites but not at others has been a puzzling observation.

Objectives

To demonstrate that long-term chlorinated solvent exposure affected the compliment of RDase genes in resident *Dhc* strains and resulted in dechlorinator phenotypes that efficiently utilize the available chlorinated solvent electron acceptors.

Approach

Metagenome sequences were generated from Third Creek (Knoxville, TN) sediments collected at a chlorinated solvent-contaminated site, as well as three upstream locations without reported chlorinated solvent contamination. To test for the activity of *Dhc*, sediment microcosms were established in defined mineral salts medium amended with lactate as electron donor and tetrachloroethene (PCE) as electron acceptor.

Results and Conclusions

PCE was detoxified to ethene in microcosms established with sediments collected from the chlorinated solvent-impacted site and two upstream locations. No PCE dechlorination occurred in microcosms established with material from the third upstream sampling location. *Dhc* 16S rRNA genes were detected in all sediment samples. The *vcrA* and *bvcA* genes implicated in chlorinated ethene reductive dechlorination were only detected in the sediment samples that yielded ethene-producing microcosms. *Dhc* contigs were recovered from all metagenome datasets, including the sediment that showed no reductive dechlorination activity. Comparative metagenome analyses are used to determine the key genomic features (e.g., RDase gene complement) that distinguish *Dhc* strains present in different locations of Third Creek. The 70-year history of localized chlorinated solvent contamination in Third Creek offers opportunities to unravel RDase gene acquisition and community adaptations that lead to the evolution of communities that efficiently attenuate toxic chlorinated solvents in the sediment.

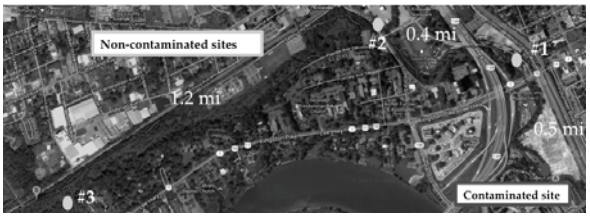

Fig. 1. Sampling locations, Third Creek.

P77

The influence of Acacia invasion and clearing methods on bacterial communities in Fynbos soil

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Soil microbes are important role-players in the functioning of natural ecosystems. Of particular interest is the influence of disturbances such as agriculture practices on the diversity and structure of soil bacterial communities. Disturbances also include the invasion of alien plant species in natural ecosystems. Species such as *Acacia mearnsii* and *A. saligna,* have an extremely high invasive potential and is now a common site in the fynbos habitat. Acacias often form monocultures in the oligotrophic soils of the Fynbos and completely displace native vegetation. Clearing of invasive Acacias are done in an attempt to restore the natural vegetation. This study aimed to determine the effect of Acacias on the soil bacterial community and the effect of the slash and burn clearing method on the soil bacterial community. The experimental treatment sites consisted of 50 one-hectare plots which were slashed and burned. Soil samples were taken from natural Fynbos reference sites, invaded control sites and from experimental sites before and after slashing and burning of invasive trees. Sampling was repeated every two weeks for three months, and once a month thereafter. This study employed a molecular approach, which included targeted amplicon sequencing of the variable region V5 of the 16S rRNA gene using the Ion Torrent PGM. The high throughput sequencing data was quality filtered and analysed using MOTHUR. The chemical properties of the soil samples were determined which included available nitrogen. Using multivariate analysis the link between Acacia invasion, soil properties and the bacterial community composition was investigated. The results revealed significant differences in the community structure between Acacia stands and natural Fynbos sites, similar to what was found in a previous study. The burning of sites resulted in a highly variable bacterial community composition compared to the unburned control sites. Burned sites were characterised by an increase in available nitrogen and this correlated with changes in the bacterial community structure. The study thus showed that both the invasion itself and the treatment had a significant effect on the soil bacterial community.

P78

Conceptual and methodological framework to manage microbial robustness using molecular systems synecology: Applications for the biodegradation of contaminants of emerging concern

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Microbial communities lie at the heart of any biological processes that contribute to ecosystem functioning and have been playing a crucial role in elemental biogeochemical cycling for more than 4.5 billion years. However, a brief survey of environmental history of the twentieth and twenty-first centuries reveals that microorganisms have been consistently challenged by a wide range of man-made recalcitrant chemicals. Although biodegradative activities of a microbial community cannot be exclusively attributed to any of the individual members, autecological (i.e., population-centred) approaches have dominated biodegradation studies of anthropogenic compounds.

With the emergence of high-throughput molecular techniques, there is a compelling impetus to move from individual-based analysis to synecological (i.e., community-centred) approaches for the study of microbial degradation processes within the context of natural environments. The molecular systems synecology approach developed in this work seeks to achieve a systems-level understanding of community functioning and a whole picture of intricate interactions within microbial communities involved in the turnover of contaminants of emerging concern. Using microbial degradation kinetics quantified in environmentally-relevant, continuous flow bioreactors and community-level omics data sets gained from targeted and shotgun illumina sequencing, community structure and function as well as microbial robustness in the presence of different types of disturbance are decoded for diverse microbial communities such as dehalorespiring mixed cultures. The use of stable isotope probing (SIP) techniques and targeted sequencing of key functional genes enables to capture the role of individual community members, their influence on the system and the abundance of functionally redundant species. The combination of SIP and shotgun sequencing is also used to decode metagenomes characterized by lower complexity and higher resolution/coverage for specific active functional guilds (including low-abundance community members). The proposed framework can be used to shed light on the governing principles of microbial community robustness and the mechanisms for resistance and resilience in response to environmental stresses at the system level.

P79
Analysis of methane production and methanogenic community across the rice cultivars

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Introduction
Methane emission rate varies considerably among rice ecosystems having different rice cultivars. The information as to how cultivar affects rhizospheric methanogenic community structure is essential to understand the microbial ecology of CH_4 emission from paddy fields.

Objectives
Effects of rice cultivar on CH_4 production and associated methanogenic diversity were studied in tropical rice fields.

Materials and Methods
Six different rice cultivars (three upland cultivars: IDR 763, HUR 3022, Sahbhagi; three lowland cultivars: Swarna sub 1, MTU 7029, BPT 5204) were chosen for the present study from Agriculture farm of BHU, Varanasi, India. NH_4^+-N content and plant biomass were determined using standard protocol. CH_4 production potential was measured using gas chromatograph. Population size of methanogens was determined by real-time qPCR targeting *mcr*A gene fragments. Diversity analysis was done by denaturing gradient gel electrophoresis (DGGE) targeting 16S rRNA genes.

Results
NH_4^+-N content did not vary significantly among upland and lowland cultivars but plant biomass varied significantly. Population size of methanogens varied among cultivars (upland: 1.7-4.0×10^5 copies g^{-1} dws; lowland: 60.4-97.6×10^5 copies g^{-1} dws). Diversity analysis revealed presence of Methanocellales, Methanobacteriales, Methanomicrobiales, *Methanosaetaceae* and *Methanosarcinaceae* in all cultivars samples but with varying composition. A pattern of difference in methane production potential after 20 days of incubation was in the order Sahbhagi < HUR 3022 < IDR 763 < Swarna sub 1 < MTU 7029 < BPT 5204. Cumulative CH_4 production varied from 295.6 to 389.1 µg CH_4 g^{-1} dws (upland) and 444.1 to 684.3 µg CH_4 g^{-1} dws (lowland).

Conclusion
Rice cultivars with different plant biomass offer selective favourable niche for the prevalence of methanogenic archaeal community that impart differential effect on the methane production potential of soils.

P80
Solubilisation of iron ore mineral by a fungus

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This study focussed on direct and indirect bioleaching potentials of fungal isolates indigenous to iron ore minerals. The experiment involved the use of potential mineral-solubilising fungi that were successfully isolated from the surfaces of iron ore minerals. Four isolates were obtained and identified by molecular and phylogenetic methods as close relatives of three different genera, namely *Penicillium* (for isolate FO), *Alternaria* (for isolates SFC2 and KFC1) and *Epicoccum* (for isolate SFC2B). An insoluble form of phosphate-tricalcium phosphate ($Ca_3(PO_4)_2$) was utilised in phosphate-solubilising experiments to confirm isolate FO as the only phosphate solubiliser. The bioleaching capabilities of this fungus and its spent liquid medium were tested and compared using two types of iron ore materials, conglomerate and shale, from the Sishen Iron Ore Mine as sources of potassium (K) and phosphorus (P). The spent liquid medium removed more K (a maximum of 32.94% removal, from conglomerate), than the fungus (a maximum of 21.36% removal, from shale). However, the fungus removed more P (a maximum of 58.33% removal, from conglomerate) than the spent liquid medium (a maximum of 29.25% removal, from conglomerate). The results also indicated a potential relationship between the removal of K or P and the production of organic acids by the fungus. The high production of gluconic acid could be linked to the ability of the fungus to reduce K and P. Other acids produced by the fungus in lower concentrations included acetic, citric and maleic acids. In addition, particle size and iron ore type were also shown to have significant effects on the removal of potassium and phosphorus from the iron ore minerals. It was concluded that the spent liquid medium from the fungal isolate FO has the potential for being used in the biobeneficiation of iron ore minerals.

P81
Increasing management intensity reduces the diversity of arbuscular mycorrhizal fungi on Arabica coffee (*Coffea arabica*) in its Ethiopian center of origin

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Arbuscular mycorrhizal fungi (AMF) associate with coffee and enhance plant productivity. The AMF community present in roots of Ethiopian coffee plants is investigated. *Coffea arabica* is an important economic crop which is endemic to the Ethiopian highlands, where it is an understory shrub of the Afromontane

rainforest. *Coffea arabica* roots from different management systems (forest, semi-forest, semi-plantation, plantation and home garden) were analyzed. These management systems represent a gradient of increasing human intensification. We used 454 pyrosequencing of the SSU rRNA gene to characterize AMF communities. Sequencing produced 10085 AMF sequences which generated operational taxonomic units (OTU's) to evaluate the AMF community composition: 36 unique AMF OTU's were detected for 42 root samples. OTU's were found from the four Glomeromycota orders and eight families. While AMF communities in the five management systems showed particular features, suggesting community dissimilarity, the five most abundant OTU's were shared between all management types. The environment with no human disturbance (forest) showed a very high AMF biodiversity while those subjected to human input had a lower diversity where plantations had a significant lower number of OTU's and sequences compared to forest. Moreover, community composition of AMF was significantly altered from forest to plantation coffee. Our results indicate that AMF biodiversity is influenced by human intensification, illustrating a gradient of AMF communities which reflects the management gradient. Extended knowledge on the benefits of AMF diversity in this economic crop in relation to environmental factors can prove far-reaching.

P82

Assessment of forest soil bacterial community structure and physiological characterization of key bacterial taxa

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Coniferous forests represent a large biome in the boreal zones as well as a climax vegetation zone in the mountains of the northern temperate zone. They represent an important C sink and are thus of global importance.

The main objective of the present study was to comparatively evaluate the bacterial community structure, in litter and humic horizons, from a *Picea abies* forest soil and to isolate, identify and characterize the most abundant taxa with respect to their involvement in environmental processes.

In order to obtain high accurate soil biodiversity results, 454 barcoded pyrosequencing was applied. Furthermore, five different solid media were used with the purpose of isolating the maximum number of different heterotrophic bacteria with a specific goal to enrich for slow growing taxa. Biolog GN2 plates, MicroResp and enzymatic activity quantification were used for further physiological characterization of the strains.

Pyrosequencing results revealed that regardless of the strong acidic pH of soil, a highly diverse bacterial community was present in both horizons. Indeed, bacterial sequences belonged to 17 phyla which were recorded with abundances over 0.1%. *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were dominant in litter and humic horizons, comprising 74-88% of all sequences. Moreover, as in other low pH soils, *Acidobacteria* was the predominant phylum especially in the humic horizon, accounting for more than 40% of all sequences. More than 1100 bacterial were isolated from the forest soil belonging to 400 OTUs at a 97% similarity threshold. Among them, 63 OTUs corresponded to taxa recovered by pyrosequencing data and were subject to subsequent physiological characterization. *Proteobacteria* was the predominant phylum among the isolated bacteria (62%) and also two different strains of *Acidobacteria* were obtained.

This study confirms a strong bias among the analyses of bacterial community based on molecular approaches versus culture-based approaches.

P83

Isolation of phenol-catabolic genes by cultivation-independent functional screening from metagenome of soil artificially polluted by aromatic hydrocarbons

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Introduction

Many genes for degradation of aromatic hydrocarbons (AHs) have been isolated from pure-cultured bacteria capable to utilize AHs as sole sources of carbon and energy. However, only less than 1% of microorganisms in the environment are readily culturable under the laboratory conditions, and abundant unculturable microorganisms are expected to potentially encode the novel catalytic activities.

Objectives

To efficiently obtain genes for aromatic ring-hydroxylating oxygenases, the key enzymes for degradation of AHs, a cultivation-independent approach was used.

Materials and Methods

A soil was polluted artificially and simultaneously by four AH compounds and incubated for 49 days at 25°C. Metagenomic DNA extracted from the soil was cloned to a broad-host-range cosmid vector to construct in *Escherichia coli* the metagenomic DNA library with 5.2-Gb inserted fragments in total. The resultant library was introduced into *Pseudomonas putida* G7 derivative carrying a naphthalene dioxygenase mutant of NAH7 (a catabolic plasmid encoding all the enzymes necessary for the complete degradation of naphthalene). Indigo-forming clones on the indole-containing agar plates were screened. Because indole is a common substrate for various aromatic ring-hydroxylating oxygenases, the positive clones were expected to harbor the cosmids carrying a part or whole set of genes for aromatic ring-hydroxylating oxygenases.

Results

A total of 16 positive clones were obtained, and one of cosmids from the positive clones was sequenced. In the 24-kb insert, open reading frames (ORFs) were found that showed 65-96% identities at amino acid level with whole set of genes for phenol hydroxylase from *Cupriavidus nector* JMP134. The clone harboring this cosmid indeed showed the phenol-degrading activity. The ORFs were more abundant in the polluted soil metagenome than in the non-polluted control one.

Conclusion

We successfully obtained novel genes for phenol degradation from the soil metagenome by using cultivation-independent functional screening approach.

P84
Metagenomic analysis using long 16S amplicons and the Roche 454 GS FLX+ platform

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Metagenomic analyses using 16S rRNA or other amplicons for analysis of microbial communities in environmental or human associated samples are mostly limited to short reads of 100-500 bp due to the maximal read length of the applied sequencing platforms. The release of the 454 GS FLX+ sequencing platform enables the combination of reads up to 1 kb with high throughput sequencing. In this study we assessed and optimized the usability of this platform in sequencing long amplicon libraries for metagenomic analyses.

For this purpose we used a mock community with species derived from various habitats (e.g. human associated, soil, aquatic) for generating libraries comprising long amplicons. We used Lib-L fusion primers which amplified products between 600 bp and 1 kb and spanning multiple 16S variable regions (e.g. V4 to V9 for the 1 kb amplicon).

Several optimizations including library purification methods and emulsion PCR titrations were effectively tested. Libraries containing these amplicons were successfully sequenced, with the modal read length reaching the total amplicon length of the 1 kb amplicon. Furthermore for this longest library, more than 97% of the reads were long enough to span more than four consecutive variable regions while up to 50% of the reads contained all 6 amplified variable regions.

The combination of multiple variable regions in one single read leads to a better phylogenetic assignment of the observed taxonomic units. The generation of such long amplicon reads using a high throughput sequencing platform enables a more precise insight into the analyzed community thus opening new perspectives for metagenomic analyses.

P85
Mining for bacterial chitinases - from a chitin-agar plate to the screening of metagenomes

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Chitin is one of the most abundant biopolymer on Earth. Although bacteria do not produce chitin, they use it as a source of nitrogen and carbon. The majority of bacterial chitinases are assigned to family18 of the glycosyl hydrolases, in particular to type A encoded by *chiA*. The main objective of this study was to assess the chitin degradation potential of the microbial communities in different terrestrial and aquatic habitats. We examined the bacterial communities of ten different habitats, including aquatic and terrestrial ones. Two large soil metagenomic libraries were screened for the presence of chitinase genes. Paired-end sequencing of fosmids was applied. *De novo* assembly and annotation allowed the identification of a new bacterial chitinase. Metagenomic investigation of an agricultural soil amended with chitin offered new insights on soil suppressiveness towards plant pathogens.

P86
Rice endophytes and their functions

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Beneficial microorganisms that live within crop tissues, or endophytes, and can promote plant growth and disease resistance are of high potential for agricultural purposes. This research has completed the characterization of ~60 culture-dependent rice endophytes from the root, stem and root tissues of 4 common native rice cultivars. In addition, we have performed a DGGE analysis of the microflora composition of culture-independent rice endophytes and will continue to identify the DGGE bands with sequencing approach. Among the culture-dependent rice endophytes, approximately 30% of the microbial strains possess phosphate-solubilizing capability, 25% with siderophore producing and 55% with IAA producing capabilities. In addition, 5 endophytes showed antagonistic effects on several common crop fungal diseases. These endophytes that possess Plant Growth Promoting Substance (PGPS) producing capabilities were arbitrarily selected for re-colonization assay into rice. Results of 30 days after inoculation in seeds indicated that 8 strains could significantly increase plant fresh weight, while 11 strains could increase plant height. Amongst, strain number 4, 13, 29, 79 and 88 could individually increase both plant fresh weight and height, which deserves further investigation. The establishment of this rice endophytic database will provide the academy and the industry a platform for the functional application of novel endophytes. These findings will benefit rice cultivation in the subtropical and tropical regions.

P87
Co-occurrences of fungi and bacteria at biogeochemical interfaces in aged artificial soils

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Soil microbial ecology is strongly influenced by the presence of high variety of spatially heterogeneous biogeochemical interfaces (BGI). In this study we investigated the influence of BGI on their colonization by soil bacteria and fungi in four aged artificial soils during the degradation of plant litter.

The soils contained quartz sand and silt in combination either with (i) montmorillonite, (ii) illite, or mixtures of (iii) montmorillonite and charcoal or (iv) illite and ferrihydrite (Pronk *et al.*, 2012). All four soil mixtures were inoculated with a natural microbial inoculum which was water-extracted from a Luvisol and then aged for two years. The soils were then amended with maize-potato litter (1 % (w/w)) and incubated for 7, 21 and 63 days. Their microbial communities were analyzed using terminal restriction fragment length polymorphism analysis (T-RFLP) and fungal ribosomal intergenic spacer analysis (F-ARISA).

Soil aging and addition of plant litter resulted in distinct shifts of bacterial and fungal community patterns in all soils studied.

Community changes of fungi and bacteria induced by aging were most explicit in presence of charcoal yet not of ferrihydrite, which showed impact on bacterial communities only. Plant litter addition, by contrast, resulted in distinct reaction of all microbial communities in all soils.

Our data hence suggest that BGI are drivers (for bacteria and fungi both) during colonization of new habitats, as well as their responses to environmental changes, such as the addition of plant litter substrates. Preliminary statistical correlations of soil composition with bacterial or fungal communities further indicate significant BGI-specific fungus-bacteria interactions during BGI colonization. Shared niche preferences and helper-effects (e.g. preferential dispersal along mycelia ("fungal highways")) hence might foster soil type dependent community patterns.

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P88

Phylogenetic analysis of the prokaryotic community in nitrogen-treated soils of a tropical montane mountain ecosystem in South Ecuador.

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In a Nutrient Manipulation Experiment (NUMEX) research plots at different elevations (1000m, 2000m, 3000m) in the National park Podocarpus in South Ecuador have been treated with nitrogen over 4 years. Finally, gross N mineralization (increased) microbial biomass and ammonium immobilization (decreased) and gross nitrification (increased) were measured.

The study aims to assess the prokaryotic community in correlation with nitrogen-cycle related data.

The prokaryotic community has been assessed from nitrogen-treated plots and compared with untreated plots from all three elevations. Furthermore, a soil depth profile comprising soil depth from 0 - 15 cm, 15 - 30 cm and 30 - 50 cm were analyzed. The community has been analyzed by using the 16S rRNA gene. Therefore, amplicons of the 16S rRNA region V3-V5 were generated using primers specific for Bacteria as well as for Archaea. Amplicons were sequenced and the sequence datasets are processed by bioinformatic tools and analyzed together with environmental as well as nitrogen-cycle related data.

Due to chronic nitrogen addition there might be a change in the prokaryotic community composition compared to the untreated plots. For example, the abundance of *Cyanobacteria*, *Chloroflexi* and *Nitrospirae* tend to increase, whereas *Alphaproteobacteria* seem to be less abundant in the nitrogen-treated plots. In general, *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* are highly abundant in both plot types.

P89

Soil bacterial community succession along a salt marsh chronosequence: insights into temporal niche segregation promoting phylotypes co-existence

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Introduction

Understanding the factors that modulate changes in community composition along environmental gradients is a central theme in ecology. Despite investigated in plants for centuries, ecological succession of microbial communities remains mostly elusive.

Objectives

We focused on three main ecological aspects of bacterial succession: the relationship between abiotic factors and community succession (allogenic succession); temporal dynamics in phylogenetic community composition; and phylotype co-assembly, accessed by co-occurrence network analysis at each stage of succession (autogenic succession).

Materials and Methods

Samples were collected at four sampling-times in 2012, in triplicated plots along five established stages of succession (0 to 105 years) in the salt marsh chronosequence of the island of Schiermonnikoog, NL. Bacterial communities were accessed by qPCR (abundance) and 454-pyrosequencing (diversity), targeting for the 16S rRNA gene. Sequences were processed using QIIME and the R environment.

Results

Despite the 10-fold lower 16S rRNA gene abundance, the youngest sites held higher α-diversity and phylogenetic diversity than soils from late succession. This could be partially attributed to the temporal variations in phylogenetic β-diversity, detectible at a monthly scale for soils at initial stages (0 and 5 years), but not for intermediate and late stages. Allogenic succession played an important role in assembling bacterial communities, being mostly governed by shifts in the physical structure of soil, soil pH and salinity, explaining together 84.5% of the variation. Furthermore, analysis of phylotype co-occurrence within sites suggested a minor role of autogenic succession, possibly occurring at finer scales. Remarkably, patterns of species co-occurrence were highly complex at the early stages of succession, as indicated by larger networks with high number of modules, providing evidence for the importance of temporal niche partitioning in promoting phylotype co-existence.

Conclusion

By unravelling bacterial network dynamics, we could provide evidence that the significance of temporal niche segregation at early stages exceeds the influence of the spatial niche distribution in promoting species coexistence at the late stage of succession.

P90

Dependence of soil bacterial community composition and diversity on land use types and management regimes

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Soil inhabiting bacteria are key players in environmental processes. Their community composition is influenced by a number of biotic and abiotic factors. Management intensity is known to exhibit a negative effect on diversity at higher trophic levels. Some reports indicated that bacterial diversity in managed systems is lower than in the corresponding natural systems, but also the opposite has been found. However, in many studies sampling design and survey sizes did not allow statistically robust assessment of management influence on bacterial diversity and community composition.

We are currently assessing bacterial community composition and diversity of 300 soil samples derived from the German Biodiversity Exploratories by analysis of bar-coded 16S rRNA gene amplicons. The soil samples encompass the different land use types grassland and forest and 13 different management regimes. The V3-V5 region of the 16S rRNA gene, which is established and widely applied as marker gene for phylogenetic classification of bacteria, was analyzed. The dataset comprises at least 7,000 high quality 16S rRNA gene sequences with an average read-length of approximately 570 bp per sample.

Preliminary analyses of 150 samples showed significant differences in community structure on the phylum level between grasslands and forests as well as an impact of soil edaphic properties on community composition and diversity. However, management regime does not seem to significantly influence bacterial diversity at phylum level. The bacterial community seems to be able to adapt to most changes introduced into the system, preventing a loss of bacterial diversity and thus a loss in ecosystem functioning. Adaptations require a shift in bacterial community composition, but the diversity remains intact at phylum level. These preliminary analyses support findings that soil parameters seem to play a major role in driving bacterial community composition and diversity. Management intensity might not be relevant to bacterial community structure and diversity, as it is at higher trophic levels. Nevertheless an influence of management regime on composition and diversity of certain bacterial groups might be encountered during the currently performed analysis at higher taxonomic resolution.

P91

Integrated bioinformatics analysis on the soil metagenome

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Question

As is well known, soil is a complex ecosystem harboring the most prokaryotic biodiversity on the Earth. In recent years, the advent of high-throughput sequencing techniques has greatly facilitated the progress of soil ecological studies. However, how to effectively understand the underlying biological features of large-scale sequencing data is a new challenge.

Methods

In the present study, we collected 33 metagenomes from diverse soil sites (i.e. grassland, forest soil, desert, Arctic soil, and mangrove sediment) and integrated some state-of-the-art computational tools to explore the phylogenetic and functional characterizations of the microbial communities in soil.

Results

Microbial composition and metabolic potential in soils were comprehensively illustrated at the metagenomic level. A spectrum of metagenomic biomarkers containing 46 taxa and 34 metabolic modules were detected to be significantly differential that could be used as indicators to distinguish at least one of five soil communities. The co-occurrence associations between complex microbial compositions and functions were inferred by network-based approaches.

Conclusions

Our results together with the established bioinformatic pipelines should provide a foundation for future research into the relation between soil biodiversity and ecosystem function.

P92

Rhizosphere microbial community structure of different parental types of *Arabidopsis thaliana* MAGIC lines

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Plants can interact with microbes through changes in the composition and concentration of root exudates. The composition of bacterial communities in the rhizosphere and endosphere are distinct between plant species, developmental stages and even ecotypes. However, while studying the microbial community in the rhizosphere of *Arabidospsis thaliana* most works were focusing on the bacterial part of the microbial community (Lundberg et al. 2012, Bulgarelli et al. 2012).

In this study, which is part of the joint project "Chemical Communication in the Rhizosphere", we are investigating the rhizosphere and endosphere fungal and bacterial communities of 19 parental types of the MAGIC lines of *Arabidopsis thaliana* (Kover et al. 2009). The parental types were cultivated in pots under controlled phytochamber conditions on two soil types. When the plants reached an early flowering stage roots, rhizosphere and bulk soil were collected. Subsequently microbial genomic DNA and RNA were extracted. The genomic DNA and cDNA samples are currently being analyzed for potential and active fungal and bacterial communities using paired-end Illumina sequencing approach.

The project concept and preliminary results on the rhizosphere microbial communities of the different parental lines and their correlation with the root exudation patterns will be presented.

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P93

Response of AM fungal communities to land-use regimes of three biodiversity exploratories in Germany

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Soil fungi, especially members of the phylum Glomeromycota, are important components of grassland ecosystems providing a direct link between the plant root and the soil environment through the formation of arbuscular mycorrhiza (AM). This symbiosis enables a direct exchange of soil minerals against photoassimilates with more than 80% of land plants. Consequently the diversity and community composition of AM fungi has been increasingly studied in different ecosystems. But there is still a need to assess the impact of land-use on the AM fungal diversity and community composition in geographically separated grassland ecosystems.

Within the frame of the interdisciplinary joint project "Biodiversity Exploratories", which includes 150 experimental grassland plots located in three geographic regions representing broad gradients of land-use intensity, we are currently investigating the change in the potential and active AM fungal communities under variable land-use regimes by using 454 sequencing of the 18S rDNA and cDNA target region respectively.

Our results indicated a significant difference in the AM fungal diversity and species richness among the three study sites. Furthermore, we will present results on the impact of geographic location, land-use intensity as well as soil and plant parameters on the AM fungal community composition.

P94

Metagenomic approach to identify major cellulase families in agricultural soils under different management practices

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Plant residues play an important role in agriculture to maintain carbon stocks in soil and improve soil fertility. During the degradation of plant litter glycoside hydrolases (GH) and carbohydrate binding modules (CBM) play a major role, as most plant residues contain large amounts of cellulose. We investigated the influence of different forms of tillage and N fertilizer on the enzymatic activity of cellulases. To further assess the abundance and diversity of different GH and CBM families we used a metagenomic approach. Soil samples were taken after the harvest from an experimental site in southern Germany, where different forms of tillage and nitrogen application have been tested since 1992.

Our data indicate that different forms of tillage significantly influenced potential cellobiohydrolase- and ß-glucosidase-activities. Soils under reduced tillage showed a higher enzymatic activity than soils under normal tillage. In accordance, microbial biomass was higher in soils under reduced tillage compared to soils where normal tillage practice was used. The influence of different fertilization intensities on enzymatic activities and microbial biomass was less pronounced. Analysis of total metagenomic data or GH families did not reveal any prominent differences between tillage treatments. Prediction of exo-, endoglucanases and ß-glucosidases revealed GH families 1, 3 and 5 and CBM 4, 6, 9 and F5/8 type C domains as the most abundant families in soil and proteobacteria, actinobacteria and firmicutes as the dominant phyla that harbour these genes.

Together these data show that reduced tillage increases both microbial biomass and enzymatic activity of cellulases but does not change the diversity of cellulase families.

P95

Fungal community function in chronosequence of land abandonment

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Besides phylogenetic diversity, the functional diversity of soil micro-organisms is important. The presence of genes involved in degradation of organic matter influences competitive interactions among organisms, which shape community composition, as well as modulate the decay rate of plant detritus and the subsequent formation of soil organic matter. In this experiment a set of molecular functional parameters were tested and the functioning of soil fungal communities along a chronosequence of land abandonment from agriculture was evaluated. The questions asked in this research are i) if the soils from different land abandonment types differ in their functioning, ii) if there is a detectable relationship between fungal phylogenetic diversity and functional diversity and iii) what is the degree of functional complementarity among soil fungi. The hypothesis are that fungal diversity positively correlates with fungal community function and most diverse community of fungi is found in the fields that are abandoned the longest time (lowest disturbance).

The genes studied included fungal Mn-peroxidase, laccase, nitrate reductase, glucose oxidase, cellobiosedehydrogenase, oxalate decarboxylase, heme-thiolate peroxidase and 12 glucosyl hydrolase (GH) families. The diversity and composition of these genes were evaluated from the soils collected along the chronosequence using cloning and (Sanger) sequencing approach. Approximately 75 unique gene types were identified per gene and evaluated using UniFrac for differences between soils and time since abandonment.

Six of the genes analysed revealed significant differences between fields abandoned for shorter and longer time while 13 genes showed no consistent pattern between the treatments. However, all genes revealed significant differences between some of the fields studied, and this could be related to other soil parameters such as pH, organic matter and the amount of available nitrogen.

Contrary to our hypothesis, most of the genes were equally abundant in all the fields and only in the case of few genes, large differences were found between the abandonment regimes.

Furthermore, these genes were not often related to the decomposition of more recalcitrant matter but rather involved in degradation of cellulose and hemicellulose (i.e. glycocyl hydrolases).

P96
Phylogenetic and functional characterization of microbial communities in mesophilic compost soils harbouring different organic matters

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Composting is the degradation of organic matter by aerobic microbes resulting in nutrient-rich humus. The process can be distinguished into different phases in which temperatures can rise up to 80 °C.

In this study, compost soils ranging from 20˚C to 55 ˚C were collected from 3 aerated static piles at a compost plant in Göttingen, Germany. The main substrates were grass and hay for pile 1 (S1), coniferes for pile 2(S2), and household waste mixture for pile 3(S3).To gain comprehensive insights into the inherent and active diversity of microbial communities in the samples, DNA and RNA were extracted, processed, and used as templates to amplify the V3-V5 region of genes for the bacterial16S rRNA. Subsequently, the PCR products were sequenced applying next-generation sequencing and sequence data were analyzed with the Qiime software package. In addition, metagenomic libraries were constructed and functionally screened for industrial relevant enzymes.

On DNA level, the phyla *Actinobacteria* and *Firmicutes* were dominant in samples of S1, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* dominated in samples of S2 and S3. The relative abundances of *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Gammaproteobacteria* decreased with rising temperatures among samples of S1 or S3. Clustering analysis showed that the bacterial communities were mainly grouped according to the substrate, rather than temperature. In comparison, the study of active members of the communities revealed that the phyla *Actinobacteria* and *Firmicutes* were still dominant in samples of S1 and S2, *Firmicutes* and *Gammaproteobacteria* were dominant in samples of S3. However, only *Firmicutes* had a significant increase in abundance when the temperature increased in samples of S3. As already shown on DNA level, samples from the same substrate also clustered together. Nonmetric Multidimensional Scaling (nMDS) analysis of all samples indicated that the active part of the community may have a different succession pattern comparing to the whole community. The functional screening resulted in the detection of 4 amylases, 5 xylanases, and 3 proteases active clones.

P97
Recovery of soil microbial populations, function, and community composition following reclamation of a lignite surface mine

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Lignite surface mines are typically reclaimed by backfilling with previously removed soil; however, this process destroys the original soil structure thus disrupting its biological properties. A study was conducted to determine the amount of time required for microbial abundance, function, and community composition to recover in a 40-year chronosequence (0, 5, 10, 15, 20, 30, and 40 years) of reclaimed mine soils in Texas, USA. Microbial abundance was determined by measuring microbial biomass levels and using qPCR targeting bacteria (16S rRNA) and fungi (ITS). Microbial functional activity was determined by measuring C and N mineralization rates. Microbial community composition was determined using 16S rRNA gene pyrosequencing and GeoChip functional gene microarrays. Soil microbial biomass levels and C and N mineralization required 15 to 20 years, following reclamation, to equal levels in a nearby, un-mined reference soil (UM). Likewise, numbers of bacteria and fungi (as determined with qPCR) recovered within 20 years. The soil bacterial communities at all sites were dominated by *Actinobacteria, Acidobacteria*, and *Proteobacteria.* However, community composition in the reclaimed sites did not match the UM site even after 40 years of reclamation. Interestingly, the communities in the 10- and 15-year reclamation sites were more similar to the UM site than the 30- and 40-year post-reclamation sites were. This indicates that the communities initially became more similar to the UM site, up to around 15 years, and then deviated into different communities. Since this corresponded with the recovery of major soil processes, this suggests that the functional redundancy of the microbial community contributed to the recovery of soil ecosystem functions, even though the community did not return to its original composition.

P98
Structural and functional diversity of biodegradative populations in soil polluted by aromatics

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Being the largest reservoir of microbial diversity, soil harbors many populations capable of degrading complex organic compounds, including pollutants of natural or anthropogenic origin. Diversity of these populations had originally been assessed through cultivation which favors only some taxa. In order to describe and understand all specific organisms, genes, and mechanisms involved in biodegradation, culture-independent methods need to be employed that directly link metabolic capability to phylogenetic and metagenomic information within a community context. Our research is aimed at diversity of prokaryotic degradative populations in aromatics-polluted soil. Major objectives are the identification of such populations and investigation of their biodegradative genes. In order to reach these goals, we couple stable isotope probing with 16S rRNA and functional gene pyrosequencing, specifically those encoding dioxygenases hydroxylating the aromatic ring. Sequence information is used for targeted mining of functional genes from the metagenome or DNA shuffling experiments, construction of transgenic strains bearing the genes, and determination of substrate specificity of respective enzymes.
Our results show that: (i) biodegradative populations cluster mostly with Proteobacteria, and in addition to previously described taxa (*Pseudomonas, Burkholderia, Pandoraea, Comamonas, Rhodanobacter,* etc.) include novel taxa (*Rudaea*- and *Skermanella*-related); (ii) these populations are often associated with the degradation of multiple aromatic substrates (e.g. biphenyl,

benzoate, and naphthalene); (iii) different dioxygenases evolve in contaminated and pristine environments, showing the probable functional speciation; (iv) novel dioxygenases can be retrieved from stable isotope labeled metagenomes. Overall, our findings bring insight into the identity and metabolic activities of bacteria metabolizing aromatic compounds in soil and help us better understand biodegradative processes.

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P99
Metagenomic analysis uncovered novel structure of microbial communities involved in denitrification in paddy soil

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Denitrification is one of the important processes involved in biological nitrogen transformation in paddy soil. Dissimilatory nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide (N_2O) reductase (Nos) are key enzymes in denitrification. In this study, diversity of *nir*, *nor*, and *nosZ* sequences in paddy soil metagenome was investigated.

Shotgun metagenomic sequencing of the paddy soil DNA was performed using GS-FLX titanium. All of the obtained sequences were compared against the database containing reference sequences of *nir*, *nor*, and *nosZ* obtained from Functional Gene Pipeline/Repository. Hit sequences were compared against NCBI non-redundant protein database. Public metagenomic data of the farm soil and the forest soil were also analysed in the same way.

Large majority of *nir* were related to *nir* of the bacteria belonging to the phylum *Alpha*-, *Beta*-, *Gammaproteobacteria*. Some *nor* and *nosZ* were related to denitrifiers belonging to *Alpha*-, *Beta*-, *Gammaproteobacteria*, while other were related to non-denitrifiers belonging to *Deltaproteobacteria* carrying no *nir* gene on their genome. Surprisingly, more than 30% of *nosZ* in the paddy soil metagenome were related to *nosZ* of *Anaeromyxobacter* bacteria belonging to the phylum *Deltaproteobacteria*. The *nosZ* related to that of *Anaeromyxobacter* were also found in the metagenomic data of the farm soil and forest soil, although any previous PCR-based analysis could not detect these *nosZ* sequences. *Anaeromyxobacter* bacteria isolated from our paddy soil showed potential ability to reduce exogenous N_2O to N_2 under anaerobic condition. Moreover, transcripts of *Anaerommyxobacter*'s *nosZ* were detected in the paddy soil RNA by the customized PCR-based method.

In conclusion, metagenomic analysis revealed novel structure of microbial communities involved in denitrification which could not be detected by the previous PCR-based analysis. *Anaeromyxobacter* bacteria might be important players of N_2O reduction in paddy soil.

P100
The structure of the barley bacterial microbiota

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Barley (*Hordeum vulgare*) is the world's fourth largest cereal crop and at the same time is emerging as a model to dissect the genetic and genomic basis of plant-microbe interactions. However the molecular bases of barley-microbiota relationships are still largely unknown.

The aim of this work was to gain insights on the structure of the barley rhizosphere and root bacterial microbiota within a plant domestication framework.

We have grown wild, landrace and modern barley accessions in a reference experimental soil under controlled environmental conditions in replicated experiments. We subjected total DNA preparations from unplanted soil, rhizosphere and root samples to amplicon pyrosequencing of the prokaryotic 16S rRNA gene. Upon filtering, denoising and chimera removal, pyrosequencing reads have been clustered at 97% identity for operational taxonomic units (OTUs) definition.

a-diversity indices calculated on the generated OTU table showed a significant reduction of the bacterial richness and diversity in the root-inhabiting compared to the soil communities. b-diversity analysis segregated the unplanted soil from the plant-associated bacterial assemblages. Irrespective of the tested accession, linear model analysis revealed a structural diversification of the rhizosphere and root microbiota, supported by the differential enrichment of specific OTUs. Interestingly, taxonomical assignments of these OTUs indicated that the barley microbiota comprises bacteria for which plant growth promoting activity has been reported, such as *Flavobacterium sp*., and *Rhizobium sp*., as well as bacteria with potential cellulolytic capabilities, such as *Cellvibrio sp*., and members of the order Burkholderiales. These results suggest a two-step selection process gradually differentiating the barley microbiota from the surrounding soil biome. In this model, rhizodeposition fuels an initial substrate-driven community shift in the rhizosphere, and host genotype-dependent factors contribute to the fine tuning of microbiota profiles.

Our work will set the stage for future studies aiming at the functional characterisation of the barley microbiota through metagenomic reconstruction as well as the selective isolation of its members.

P101

Determining how oxygen legacy affects the trajectories of denitrifier function and structure in soil

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Human activities have more than doubled the annual global production of reactive nitrogen (N, defined as ammonium and nitrate), and thus understanding how nitrogen cycles in natural and managed ecosystems has become a great challenge. One such challenge is that a large fraction of N is still unaccounted for, and therefore a critical question is what the fate of nitrate is. Nitrate is respired via denitrification to N_2. Therefore, denitrification is an important sink for nitrate and hence an essential process to study. One key environmental controller of denitrification is oxygen (O_2), where denitrification is thought to occur at low O_2 concentrations. Our study objective is to test how the legacy of O_2 availability in soil influences denitrification kinetics and denitrifier community structure. Distinct oxygen legacies are imposed in a laboratory setting using a remoulded grassland loamy soil from an agricultural field. The soils are pre-incubated at constant soil moisture and temperature with four contrasting O_2 profiles: 1) aerobic conditions, 2) rapid reduction to anaerobicity, 3) gradual O_2 reduction, and 4) multiple rapid oxic/anoxic spells. After the O_2 legacy effects are instilled, all soils are incubated anoxically as slurries with ample nitrate and carbon to induce denitrification. Denitrification kinetics is measured using a novel sampling robot that can quantify the production of all gaseous intermediates and N_2 from the soils. Nitrite is also measured through chemical acidification to NO. Soil DNA and mRNA is isolated at different time points throughout the slurry incubations and analyzed by metagenomic as well as metatranscriptomic approaches to asses the taxonomic trajectories of the active denitrifying communities. However, as the challenges of soil metatranscriptomics are well known, this ongoing experiment will also quantify and sequence amplicons of the denitrification functional genes and transcripts, using a similar methodology to Liu, B. et al. (abstract submitted as well). By taking this two-pronged approach of linking rates and product stoichiometry of denitrification to nucleic acid analysis we can more accurately define how O_2 legacy in soil affects the fate of reactive N and bacterial population trajectories, which may have global implications to climate change.

P102

A metagenomic study on the eubacterial populations in Alpine paleosols: Analysis based on 16S rRNA gene pyrosequencing and DGGE

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Introduction

We are working on paleosol samples collected from two locations in the French/Italian Alps at an altitude of approximately 2000+ metres. These samples are from a diverse range of micro-climate conditions, and are further subdivided into soil horizons based upon standard geological soil classification criteria: Ah, Bw, Cox and Cu horizons being typically present at each location.

There is relatively little work in the literature on the study of stable microbial populations in such sediments, and we wanted to address this issue through these experiments. Comparisons could then be made to other soil metagenomic analysis studies.

Objectives

1) To analyse the microbial populations of eubacteria through both varied depth and varied sample location in the paleosols sample sites.
2) To see if differences in population structure as observed through DGGE analysis were also demonstrated through pyrosequencing data analysis are consistent.

Materials and Methods

We have extracted DNA from different horizons by using two different extraction methods (i) MoBio soil DNA extraction kit and (ii) Rapid Method for Coextraction of DNA and RNA [1]. Density gradient gel electrophoresis (DGGE) [2] was performed on these samples and we prepared these samples for 16S rRNA pyrosequencing [3].

Results

The DGGE analysis of the samples shows that there is definitely a variation in the population in three different horizons of the soils samples. Furthermore, some geographical differences appear to emerge. Further analysis of microbial populations through the pyrosequencing data pool confirms significant variation in both the sample location and depth-related distribution of eubacteria across our sample set. The data are consistent with previous 16S rRNA pyrosequencing analysis of similar environments such as acidic permafrost deposits.

Conclusion

The conclusions from the study strongly suggest that there is indeed a link between the designated sample horizon and the structure of the microbial population observed. Some variations in the population structure at different sample locations may also be explained by the geology of the sample sites.

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P103

Understanding the edaphic drivers of cellulose-degrading guilds in an Austrian beech forest soil

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Mineral soils contain the largest pool of carbon (C) on Earth. Cellulose is one of the major constituents of this C pool since it is a key component of plant structural C. We are using biogeochemical, molecular, next generation sequencing and single-cell approaches

such as fluorescence *in situ* hybridization (FISH) and nanometer-scale secondary ion mass spectrometry (NanoSIMS) to characterize cellulose-degrading guilds over time in an Austrian beech forest soil. We hypothesize that by varying certain edaphic properties that can limit cellulose degradation, we will uncover different cellulose-degrading guilds of bacteria and fungi. Destructive soil microcosms amended with ^{13}C-cellulose were used to identify cellulolytic bacteria and fungi that respond in the presence of different types of background C and nitrogen (N) over a 25-day period. Our results indicate that addition of N in inorganic or organic forms significantly increased cellulolytic activity as measured by total $^{13}CO_2$ production and cellobiohydrolase activity. Current efforts are underway to characterize the community composition with ^{13}C-phospholipid fatty acid analysis and ^{13}C-DNA stable isotope probing combined with SSU amplicon sequencing. The ^{13}C uptake will be further analyzed at the single-cell level for population heterogeneity using the NanoSIMS. As such, we are developing and assessing methods for separating cells from soil particles for downstream FISH-NanoSIMS analysis. To that end, we compared four different cell removal treatments in conjunction with Nycodenz separation across two Austrian soils. Across our tested treatments, total recovery cells ranged from $6.5x10^8$ to $1.9x10^9$ cells/gram soil (dry wt). Changes in community structure at various stages of the cell removal process were assessed with MiSeq Illumina SSU amplicon sequencing.

The similarity of the SSU amplicon libraries generated across the different cell removal treatments and soils were assessed using the Bray-Curtis dissimilarity index and visualized using agglomerative hierarchical clustering. The end-stage Nycodenz separated communities were distinct from the starting community; we are currently identifying the populations affected by the method to be able to better interpret our future results.

P104

Soil microbial community structure and function based on N_2-fixation across different Agroecological Zones in New South Wales, Australia.

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Soil environmental gradients promote great microbial diversity[1]. Soil diversity can provide key soil ecosystem services such as biological nitrogen fixation. However, environmental factors controlling the structural and functional microbial expression along those soil gradients are not completely understood[2]. Thus, this research proposes to use PCR-TRFLP and *pyro-sequencing* analyses to determine the microbial community structure of the major soil microbial taxa (*i.e* bacteria and fungi) and the functional diversity based on N_2-fixing bacteria across different agroecological zones in New South Wales (NSW), Australia. The study area cover a north-south transect along the 550 mm rainfall isohyet, extending about 900 km from the northern (Queensland border) to the southern (Victoria border) territory of NSW. Twenty-seven sampling sites were located along the transect at a separation distance of 50 km and with a 20 km radius each. Within each site, two different land use ecosystems, natural (forest or grassland) and agricultural (rain fed crop or pasture), were selected on the same soil type based on a gamma radiometric survey. In each land use ecosystems, three soil sub-samples were taken each from 0-5 and 5-10 cm depth and kept at 4^{o}C in field conditions and then stored at -20 ^{o}C. Soil physicochemical properties were predicted by Ku-pF apparatus and near-infrared spectroscopy (NIR) previously calibrated. Soil DNA was isolated (MO BIO laboratories, Int.) to identify microbial diversity of bacteria, fungi and N_2-fixing bacteria by 16S, ITS and *nifH* gene analysis, respectively, performing PCR-TRFLP and *pyro-sequencing* methods. Expected outcomes indicate that the structural and functional diversity in Australian soils are regulated more or less by local (*e.g* soil type, land cover/use, soil C, soil pH) or global (*e.g* climate, geomorphology) environmental factors[3].

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P105

Role of bacteria-fungi interactions in hydrocarbon degradation studied in *in situ* microcosms

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Recent studies indicated the role of fungal hyphae in the mobilization of bacteria towards distant substrates promoting their degradation in unsaturated systems ('fungal highways'). Likewise hyphae were able to actively translocate contaminants ('fungal pipelines'). These findings opened the discussion whether fungal-bacterial interactions may represent an unexplored potential to remediate contaminated soils.

To assess the potential benefit of bacteria-fungi interactions for contaminant degradation, 54 microcosms containing sterile, hydrocarbon-amended soil were placed in a highly contaminated site. Degradation performance of either bacterial community versus the mixed bacterial-fungal community will be analysed. Selective microcosms permitted either no (0.1 µm pore size) or selective colonization due to exclusion by filter membranes of different pore sizes (2 µm or 30 µm, respectively) as the only contact area to the contaminated soil. Uncontaminated microcosms served as controls for hydrocarbon-unspecific colonization. Chemical losses, number of colonizing bacteria and fungi (qPCR) and microbial community patterns (T-RFLP, F-ARISA) will be analysed after one, three and six months. Metagenomic analysis and a network modelling approach are intended to reveal physical and/or metabolic networks of bacteria and fungi and to identify the interacting partners.

Data of the first time point indicate equal contaminant losses in non-colonized (control) and biotic setups. In microcosms allowing for fungal and bacterial colonization, low presence of fungi (ca. 10^3 copies/gr soil) and poor colonization of bacteria was detected. Abundance of microbial biomass in the microcosms was independent of the presence of contaminants. Fungal community fingerprints indicate similar patterns in the 2 and 30 µm setups and a significant correlation to the presence of contaminants. Moreover, only few dominant species were observed in presence of contamination. Analysis of the next time points will aim to reveal if bacteria-fungal interactions represent an advantage regarding contaminants degradation and if selective populations develop in correlation to the presence of contaminant.

P106

The genetic diversity of archaeal ammonia oxidizers drives the potential nitrification rates in Dutch agricultural soils

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Introduction
Many studies have been performed addressing the link biodiversity-ecosystem functioning, however it remains unclear to what extent the genetic diversity can be used to explain differences in ecosystems processes and to predict the overall functioning of the community.

Objectives
Using nitrification as a model process we aimed at understanding how dynamic this process and the related communities are, and to what extent the genetic diversity of archaeal ammonia oxidizers (AOAs) can be used to predict changes in the functioning of these communities.

Material and Methods
We determined the abundance and diversity of AOA in four agricultural soils (two sandy and two clayey) during the growing season in 2010. We also measured soil chemical parameters and potential nitrification activity (PNA). To further understand the changes in community composition, we performed a barcoded pyrosequencing based on the *amo*A gene.

Results
The changes in AOA community composition were found to be in the range of 50% to 72% over time, indicating a very dynamic community. Clustering the sequencing at 90% amino-acid similarity resulted in 50 representative OTUs affiliated with 5 known AOA clusters: *Nitrosotalea* cluster 1 and *Nitrososphaera* clusters 1, 3, 4 and 7. In order to determine the extent to which genetic diversity could explain community functioning, we calculated a series of diversity measures based on phylogenetic distance. The average phylogenetic distance between OTUs was significant and positive correlated to the variation in the community functioning (R2 = 0.74), indicating that more divergent communities were more productive. This percentage of variation decreased (R2 = 0.38) when using the Rao's index, which takes into account both OTU distance and abundance but was still significant. We also observed negative correlations between PNA and OTU richness, indicating that the most productive communities were dominated by few types.

Conclusion
These results indicate that PNA is likely driven by few phylogenetically distant, although abundant, AOA-affiliated types closely related to Nitrososphaera subcluster 4.1., and that phylogenetic metrics based on the archaeal amoA gene can be used to predict changes in ammonia oxidation, at least in these soils.

P107

Taxonomic profiling and metagenome analysis of a microbial community from a habitat contaminated with industrial discharges

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Introduction
Environmental habitats have lost their pristine characteristic since the beginning of industrialization on land as well as water bodies. Industrial enterprises, manufacturing dyes, paints and pigments, pharmaceuticals, chemicals, solvents and textiles, release liquid wastes containing dyes, xenobiotic compounds and many other man-made products into the environment and thus are the major cause of ground and surface water pollution in these areas.

Objectives
The aim of this study was to characterize the microbial community inhabiting an industrially contaminated site for its taxonomic profile and catabolic gene potential. Taxonomic profiling provides insights into the microbial community capable of tolerating and/or degrading xenobiotic compounds. Functional characterization leads to elucidation of the catabolic potential of the indigenous microbial community.

Materials and Methods
Soil collected from contaminated Khari-cut canal bank (N 22°57.878'; E 072°38.478'), Ahmedabad, Gujarat, India was used for metagenomic DNA preparation. Sequencing on the GS FLX System using Titanium chemistry resulted in 409,782 reads accounting for 133,529,997 bases sequence information. Metagenome sequence reads were analysed using the bioinformatics platform MetaSAMS. Taxonomic profiling was carried out by three different complementary approaches: (i) 16S rDNA, (ii) EGTs, and (iii) LCA. Metagenome reads were assigned according to GO terms, COG categories, Pfam family numbers and KEGG hits.

Results
The most abundant phylum and genus were found to be '*Proteobacteria*' and '*Pseudomonas',* respectively. Metagenome reads were mapped on sequenced microbial genomes and the highest numbers of reads were allocated to *Pseudomonas stutzeri* A1501. In total, 157,024 reads corresponded to 37,028 different KEGG hits and amongst them 11,574 reads corresponded to 131 different enzymes potentially involved in xenobiotic biodegradation.

Conclusion
Consequently, information obtained from the present study will act as a baseline, which subsequently along with other '-omic' studies help in designing future bioremediation strategies in effluent treatment plants and environmental clean-up projects.

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P109

Performance of the universal primer pairs and different sequencing chemistries (Ion Torrent vs. 454) in the 16S rRNA - based community analysis

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Both the selected primers and sequencing chemistry can potentially have an effect for the16S rRNA based microbial diversity analysis. The primers targeted to different regions of the 16S rRNA can lead to different conclusions if all of the microbial groups are not equally amplified by the selected "universal" primers, or if different 16S rRNA regions lead to different classification. Here we analyzed which primer pairs have been mostly used in the previous NGS studies for the bacterial community analyses, and tested the performance of these primers in practice. We evaluated eight pairs directed to V1-V2, V1-V3, V3-V4, V4-V5 and V6 variable regions of the 16S rRNA and compared these against the control prepared using a novel PCR

primer-independent approach. In the phylum-level no significant differences were seen in the abundance of major phyla when the three primer pairs directed to VI-V3 region were used. However, primer choice matters especially in the detection of *Planctomycetes* and low (<2 %) abundance phyla. When the results derived by the two sequencing chemistries were compared, we noticed the importance of the original sample preparation, which can cause sequencing errors and short sequences regardless of the sequencing chemistry used.

P110

Enrichment, isolation and characterization of microorganisms involved in reduction of crystalline iron(III) oxides in anoxic soil environments

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Iron oxide reduction is one of the most important electron sinks for substrate oxidation in anoxic soil environments. Using stable isotope probing of RNA, we have so far identified active acetate-oxidizing iron(III)-reducing bacteria in methanogenic paddy soils. Given that iron is the fourth abundant element on earth and acetate is the most abundant intermediate of organic matter degradation under anoxic conditions, the reduction of crystalline iron(III) oxides coupled with acetate oxidation is a noteworthy reaction in the global energy and carbon cycles. Yet little is known about the diversity and physiological characters of the microorganisms involved. Here, a combination approach of enrichment, genetic fingerprinting, isolation, and physiological characterization was implemented to describe the microbial communities capable of reducing iron(III) mineral phases in soil environments (e.g., rice paddy, forest, and wetland soils). Fifty-eight enrichments were obtained by long-term successive culture with crystalline iron(III) oxides (i.e., goethite, lepidocrocite, hematite, or magnetite) as electron acceptors and acetate as an electron donor. The T-RFLP, deep sequencing, and clone library analyses based on the 16S rRNA genes from the enrichments revealed that the hematite and goethite were highly selecting for distinctive populations of microorganisms, while all of iron(III) oxides enriched bacteria affiliated within phylogenetic groups of the *Deltaproteobacteria* (consisting mainly of *Geobacter* spp.), *Firmicutes*, *Chloroflexi*, and *Acidobacteria*. Subculturing these enrichments to a soluble NTA-iron(III) medium allowed for the isolation of 6 strains within the *Deltaproteobacteria*; 5 isolates and the other belonged to the genera *Geobacter* and *Pelobacter*, respectively. The sequence similarities of the 16S rRNA genes were 94.8%-98.1% to the closest cultured relatives. All of the isolates were iron reducers, but each exhibited the distinct ability to reduce different forms of iron(III) minerals. The results in this study demonstrate the targeted enrichment and isolation of bacteria involved in the reduction of crystalline iron(III) oxides, and the discriminative accessibility of the iron(III)-reducing bacteria to the different phases of ferric iron minerals.

P112

Methanogenic communities in soils from the Tibetan Plataeu: insights from *mcrA* amplicon pyrosequencing

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The Tibetan Plateau is the largest altitudinal permafrost unit in the world. A large amount of soil organic carbon (SOM) is preserved in the alpine grasslands on the plateau, especially in the natural wetlands which account for over 51% of the total area in China. Despite its significance, the turnover of organic matter in Tibetan environments is still unknown to many microbiologists and difficult to access. As methanogenic archaea are mainly responsible for the last step of anaerobic soil carbon turnover, the size and structure of relevant groups of methanogens is a key for carbon turnover. However, knowledge about the indigenous methanogens is very limited. Moreover, the plateau is affected by India and Asian monsoon, resulting in different temperature and moisture gradients. For this reason, our research sites were selected along the two different monsoon trajectories. Herein, we present first results on the methanogenic community structure and diversity targeted with functional *mcrA* primers (*mlas*/*mcrA*) and 454 pyrosequencing. The results show highly diverse methanogenic communities with a large proportion of yet unclassified methanogenic phylotypes including unclassified *Methanomassiliicoccus* and unclassified *Methanosarcina*-related species. More work is necessary to approach their ecophysiology. Spatial and vertical variations in the diversity and structure of the methanogenic communities could be observed in the libraries. The variations are largely associated with environmental factors such as $CaCO_3$, total organic carbon and pH. The *mcrA* amplicon pyrosequencing showed to be a promising tool to capture methanogenic taxa, especially the low-abundant species.

P113
Characterization of the Community Structure and Population Dynamics of Micropredators and Pathogens in Wastewater Treatment Plants (WWTP)

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Water shortage is a growing concern worldwide and a main problem in Israel and Palestine. Exploiting the power of microbial predators to reduce the pathogen load of discharged wastewater can be a useful approach to overcome this problem. In this DFG-funded study we analyze the dynamics of bacterial pathogens and their microbial predators in order to better understand predator-prey interactions in WWTP. While most studies on microbial predators usually focus on ciliates only, we include the complete range of micropredators, i.e. all bacterivorous protists, predatory bacteria and bacteriophages. To this end we are performing monthly sampling of several WWTP over a full year period to track seasonal community changes via a combination of high-throughput sequencing, qPCR and standard microbiological approaches. Using 454 amplicon sequencing we are currently identifying the overall bacterial (16S rRNA) and protist diversity (18S rRNA), as well as the diversity of specific bacterial predators (*Bdellovibrio*-and-like organisms). Additionally, we will use selected phage-host systems to get a better understanding of the fate of phages and their hosts during treatment processes. We incorporated environmental, chemical and biological factors to correlate our findings. Here, we would like to present the results of the 454 amplicon sequencing of the spring 2013 samples.

In conclusion, this is the first 454 sequencing study examining at the same time the dynamics of three different types of micropredators differing in their diversity, mode of predation, specificity range and efficiency in reducing the bacterial load in WWTP. Studying the microbial diversity in WWTP will answer central questions in microbial ecology and predator-prey-theory, and might lead to an improvement of the current technology. In the future, this might result in a better management of microbial resources and in a more efficient reduction of pathogenic bacteria during the wastewater treatment process.

P114
Boom Clay Borehole Water, home of a diverse microbial community

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Introduction
The Boom Clay layer located at 230 m depth under the Mol site of SCK•CEN (Belgium) is presently investigated as potential host formation for the disposal of nuclear waste. Using the HADES underground laboratory of SCK•CEN in this geological layer, the presence and activity of microbes in Boom Clay borehole water and thus potentially interacting with future nuclear waste, is addressed in this study.

Objectives
The primary aim of this microbiological study was to sample and characterize borehole water from different stratigraphic layers and to determine a common and/or abundant core bacterial community (CBC). As such CBC would find applications in laboratory experimental set-ups, this aim included the indication of preferred sites to sample borehole water to serve as CBC model inoculum. Secondly, the activity and some general metabolic pathways of members of these communities were addressed, to assess their survival and proliferation chances.

Materials and Methods
An integrated approach, using microscopy, enzymatic, molecular and cultivation-based analyses was applied to assess the diversity, activity and metabolic properties of microbial communities.

Results
A large diversity of microbes was visualized and characterized in the borehole water. Within this diversity, an abundant bacterial community (CBC), present in all Boom Clay borehole water samples was distilled, and a model inoculum was defined. The microbial community was proven to be not merely present, but also active *in situ* and viable.

Conclusion
The omnipresence of such a diverse and *in situ* active microbial community in Boom Clay borehole water samples seems surprising. Microbial contamination during piezometer installation and survival of introduced species during several years in stringent conditions are therefore considered quite credible. However, regardless of its indigenous or introduced origin, the evidence and characterisation of this ubiquitous and diverse bacterial community in the Boom Clay repository indicates the importance of microbiological research in the context of safe waste disposal and warrants more in depth assessments of clay-microbe-waste interactions.

P115
Spatial and temporal variation of the fungal metagenome away from tree trunk of spruce and beech trees

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Fungi play a significant role in the functioning of forest ecosystems serving as symbionts, pathogens, or 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 known about the relationship between soil fungi and forest tree species on the one hand, and the spatial and temporal distribution of fungal communities away from tree trunks at different soil depths on the other.

Thus we studied the soil fungal communities on a spruce (*Picea abies* L.) and a beech (*Fagus sylvatica* L.) dominated forest experimental plots of the Hainich-Dün Exploratory (Thuringia, Germany) to fill this knowledge gap. The study plots are part of a large interdisciplinary research consortium funded by the German Science Foundation (DFG) called the *German Biodiversity*

Exploratories. In both forest plots soil cores of two soil layers (0-10 cm, 10-20 cm) were sampled at a distance of 0.5 m, 1.5 m, 2.5 m, and 3.5 m away from the tree trunk from four individual trees species per plot. To evaluate seasonal dynamics, the sampling was done in May and November 2012. The fungal community was assessed using a high-throughput internal transcribed spacer (ITS) region of the ribosomal DNA pyrotag sequencing approach.

We found distinct fungal communities dominated by representatives of the phyla Basidio- and Ascomycota in the two forest ecosystems. The fungal community was found to be significantly influenced by tree species, soil depth, distance away from tree trunk and season. Using co-occurrence and network analysis we will present the relationship between the key player fungal taxa and functional groups in the two forest ecosystems.

P116
Unique functional diversity within *Sphagnum* peat bogs

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Sphagnum-dominated peat bogs represent a unique and widely distributed type of terrestrial ecosystem and strongly contribute to the global functioning of biosphere. Recent studies demonstrated that *Sphagnum* mosses are colonised by highly diverse and specific microbial communities (1, 2). To unravel this hidden functional microbial diversity in bog ecosystems, we applied an Illumina-based metagenomic approach. Through *de novo* assemby and MG-RAST annotation we revealed key biochemical pathways and adaptive strategies that constitute moss metagenome. Thus, the most dominant functional subsystems were essential for energetic and protein metabolism. A high proportion of genes involved in nitrogen and phosphorous metabolism supported the role of bacteria for nutrient supply in the extremely nutrient-poor, primarily ombrotrophic ecosystem. Genes required for environmental information processing were also abundant. Taxonomic analysis of the *Sphagnum* metagenome indicated a substantial microbial diversity. In particular the most dominant phylotypes captured by partial 16S rRNA gene sequences were assigned to Proteobacteria followed by Acidobacteria, Actinobacteria, Bacteroidetes and Verrucomicrobia. Furthermore, inter-environmental comparison revealed that the *Sphagnum* microbiome harboured highly specific genetic features distinguishing it from microbial communities of higher plants, peat soils, and aquatic systems. These unique features comprised horizontal gene transfer, stress tolerance, and interaction via quorum sensing and nutrient exchange. Overall, the *Sphagnum* microbiome possessed a highly versatile genetic potential for sustainable functioning in association with the host plants and within the peatland ecosystem.

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P117
Phylogenetic and functional diversity of soil prokaryotic communities in temperate deciduous forests with different tree species

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The application of high-throughput metagenomic and metatranscriptomic approaches have expanded our knowledge of the structural and functional microbial diversity of several different environments, including soil, a major repository of microbial life. This project focuses on the analysis of soil prokaryotic communities in the Hainich National Park, a mixed deciduous forest in Thüringia, Germany. Over time, the changes in soil microbial community composition and function were recorded in response to shifts in soil edaphic properties such as pH or nutrient concnetrations. Samples were collected from 48 mixed stands of beech, oak, lime and hornbeam in Spring, Summer and Autumn, over a period of two years. Environmental DNA and RNA was extracted and used to generate 16S rRNA gene amplicons. Preliminary DNA-based analyses showed that *Acidobacteria* are the dominant phyla in the soil, accounting for more than 30% of the total community in some plots. *Acidobacteria* are commonly associated with acidic soils; acidity that is contributed by beech tree organic matter. Despite the presence of mixed tree stands, the Hainich is primarily a beech forest, which may account for the prevalence and high abundance of this phylum. *Proteobacteria* is the second largest phylum. *Alphaproteobacteria* constitutes the largest class within the phylum, followed by *Gammaproteobacteria*, *Betaproteobacteria* and *Deltaproteobacteria* in decreasing abundances.

P118
Brazilian Microbiome Project: revealing the unexplored microbial diversity - Challenges and prospects

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Biological diversity is an important resource, not only for the environmental services it currently provides, but also for its potential as a resource in the development of new and sustainable ecosystem management tools and opportunities for bioprospecting. Despite its importance, Brazilian microbial diversity is still considered to be largely unknown, and it is clear that to maintain ecosystem dynamics, and to manage land use sustainably, it is crucial to understand the biological and functional diversity of the system. The anticipated strategic and economic benefits are related to the discovery of microorganisms for use as a source of commercially exploitable products. This possibility opens up new opportunities for the exploration of molecular bioprospecting in Brazil. The Brazilian Microbiome Project (BMP) aims to assemble a Brazilian Metagenomic Consortium/Database. At present, many metagenomic projects underway in Brazil are widely known. Our

main goal is to co-ordinate and standardize these, together with future projects.

Our challenge includes the development/dissemination of a change of paradigm related to the standards for experimental design and data analysis as well as integration with exisiting consortia and standards e.g. Earth Microbiome Project (http://earthmicrobiome.org/).
This will enable the comparison of diverse Brazilian microbiomes with others from around the world, to identify significant commonalities and differences. Currently, the BMP is organized in a committee composed by researchers from all Brazilian regions and by an International Advisory Board. This is the first attempt to collect and collate information about Brazilian microbial genetic and functional diversity in a systematic and holistic manner. New sequence data have been generated from samples collected in all Brazilian regions, however the success of the BMP depends on a massive collaborative effort of both the Brazilian and international scientific communities. Therefore, we invite all colleagues to participate in this project. There is no prioritization of specific taxonomic groups, studies could include any ecosystem, and all proposals and any help will be very welcome.

Further information and people involved
Brazilian Microbiome Project:http://brmicrobiome.org/, and links therein.

bmp
brazilian microbiome project

P119
Metatranscriptomics reveals modification of the rhizosphere microbiome by crop plants

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Introduction
Crop rotation helps boost yields but is not well understood, so there is great utility in investigating the interplay between plant roots and soil microbes. Meta'omics may give insight into the composition and activity of a rhizosphere microbiome and allow different sites or samples to be compared.

Objectives
Use metatranscriptomics to characterise and compare the composition and functional traits of bulk soil vs. the rhizosphere microbiomes of pea, oat and wheat. Investigate plant-microbe interactions via an oat mutant (*sad1*) deficient in the production of anti-fungal avenacins.

Materials and Methods
Plants were grown for 4 weeks in soil from a local field with plant-free pots as controls. RNA was extracted from each rhizosphere or control and sequenced by 454 and MiSeq. Absolute counts for assigned reads per taxa or functional category/pathway were used to compare the samples via multi-variate analysis.

Results
Eukaryote abundance in the pea and oat rhizospheres was ~5-fold greater compared to wheat and bulk soil, with pea samples enriched for fungi. Soil grown with wheat was largely unchanged.

There was a shift in the eukaryote community between the oat genotypes but little overall difference in the resulting fungal communities. Initial KEGG pathway analysis shows pea and oat sharing many functional traits but little difference between wheat and bulk soil.

Conclusions
Plants can quickly and significantly change the rhizosphere microbiome; this may be one benefit of crop rotation. The pea rhizosphere showed the most profound changes and wheat relatively little change. Using RNA-based sequencing we are continuing our functional metatranscriptomic analysis of these rhizospheres.

P120
Strategies for functional gene amplicon sequence processing

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Pyrosequencing of 16S rRNA genes have become a standard technique for prokaryotic diversity description. Many different strategies of 16S rRNA gene amplicon sequence processing have been developed that are mostly based on pre-clustering and clustering aligned sequences at a certain cutoff (usually 3%) into operational taxonomic units (OTUs). Such strategies are very efficient in masking PCR-generated and pyrosequencing errors and do not radically inflate diversity estimates. However, any insertion or deletion in functional gene sequences is fatal as it causes frameshifts in the protein sequence. Functional gene amplicon sequence processing is therefore a lot more challenging and requires cautious approaches.

Our aim was to compare different analysis strategies for functional gene amplicon sequences. Our model examples were biphenyl and benzoate dioxygenase genes amplified from both pristine and polluted soils. Strategies of sequence data processing were tested on a mock community consisting of genomic DNA of biphenyl- and benzoate-degrading bacteria harboring *bphA* and *benA* genes encoding for biphenyl and benzoate dioxygenases, respectively. Raw data processing was conducted with the use of different pipelines, such as mothur (Schloss et al., Appl. Environ. Microbiol. 2009, doi: 10.1128/AEM.01541-09) or UPARSE (Edgar, Nat. Methods 2013, doi: 10.1038/nmeth.2604), and different settings for individual steps, such as chimera elimination or sequence alignment.

Our results show that no processing results in complete elimination of pyrosequencing errors. In particular, more errors are accumulated towards the distal end of the read. Sequence length thus becomes one of the key parameters to be considered. Elimination of singleton sequences from the dataset has also proved efficient. Finally, a combination of different processing steps was chosen in order to achieve sequence clusters with representative sequences being no or minimally erroneous. Overall, this study should help more easily access nature's functional gene diversity in soil or other environmental matrices.

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P121
Different responses of soil composition-driven microbial communities to plant litter and phenanthrene in artificial soils

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Various interactions and the complexity of biogeochemical interfaces (BGIs) make a detailed understanding of soil processes difficult. Here we present a long-term artificial soil experiment providing the unique opportunity to study the structure and response of microbial communities to the model pollutant phenanthrene in the presence or absence of plant litter as a function of the soil composition. Four different artificial soils varying in type of clay mineral (illite, montmorillonite) and presence of charcoal or ferrihydrite received an identical inoculant from a natural Luvisol and sterile manure. After more than 2 years of maturation, artificial soils were spiked with phenanthrene (2 mg/g) and litter (1 wt%). After 0, 7, 21 and 63 days of incubation, total community DNA was extracted and microbial responses to phenanthrene and litter were assessed by 16S rRNA gene and ITS fragment-based analyses (denaturing gradient gel electrophoresis (DGGE), pyrosequencing, quantitative PCR).

Pyrosequencing suggested the type of clay mineral as the main driver of bacterial communities but also ferrihydrite- and charcoal-specific bacterial classes were found. By DGGE, several populations were shown to be enhanced or disappeared in response to the phenanthrene spike. In all artificial soils, dominant responders to phenanthrene were identified as *Actinobacteria. Bacteria* in illitic soils showed stronger responses than in soils containing montmorillonite. By pyrosequencing, soil composition-dependent responses to phenanthrene were identified at the genus level. Interestingly, the addition of plant litter, assumed to foster horizontal gene transfer and adaptation, affected bacterial communities but decreased their response to phenanthrene. Fungal communities responded to phenanthrene only in the presence of litter.

The present study revealed a long-term driving influence of the soil composition (minerals, charcoal) on the microbial community structure and functionality of BGIs.

P122
A cross-disciplinary soil-proteomics approach for predicting switches between hydrophilic and hydrophobic soil surface responses

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Water repellency in soils can reduce vegetation cover, accelerate water runoff, flooding and soil erosion. The presence and degree of this water repellency is controlled by many physic-chemical and biologicalfactors.

Hydrophobic proteins from soil microbes such as Streptomyces bacteria and filamentous fungi are capable of assembling extremely hydrophobic surfaces in-vitro. Our hypothesis is that such proteins play an important role in the development of water repellent soils. Preliminary findings showed that hydrophobic proteins could not be detected in wettable soils, however they were identified in moderately and severely water repellent grassland and dune soils, respectively, at medium to low soil moisture conditions. Our detection process was corroborated by extracting these hydrophobic proteins from a spiked sample of wettable soil. Further fluorescent staining with hydrophobic protein-specific stains demonstrated that there was a greater amount of hydrophobic proteins in water repellent soils than their wettable counterparts. Additionally, ournewly developed metaproteomic profiling analysissuggests that soil microbes adjust to the development of water repellency. The implications of these results will be discussed.

Combining this metadata with traditional and novel methods of soil analysis, e.g. high-resolution imaging and modelling will reveal new insights that link nano- and microscale processes with macro and field scale events.

P123
Protein-stable isotope probing (protein-SIP) identified *Pseudomonadales* and *Xanthomonadales* as active bacterial groups during leaf litter degradation in tobacco soil

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Introduction
Knowledge about the degradation process of plant-derived materials such as leaf litter is important to understand the complex mineralization cycles in nature.

Objectives
Microbial communities greatly contribute to this key process by expressing a suite of various extracellular and intracellular enzymes. However, the key players and the details of the nitrogen flux is widely unknown. Protein-SIP combine classical metaproteomics with functional information resulting from isotope incorporation into proteins thus allows tracking nutrient flux within degrading communities (Taubert et al., ISME 2012).

Materials and Methods
Soil from a tobacco field in Germany was properly mixed with leaf litter from either ^{15}N-labeled tobacco or ^{13}C-labeled corn plants as substrate. Sampling took place 7 times within two week experiments and 9 times within a 14 week experiment. Protein lysates were separated by 1-D-SDS-PAGE followed by liquid-chromatography and tandem mass-spectrometry. Mass spectra were assigned using a metagenome sequence database.

Results
10,100 distinct peptides were identified in the ^{15}N tobacco litter experiment to which the metagenome contributed to about 30%. Besides energy conversion/production and carbohydrate/amino acid metabolism, cell envelope biogenesis/outer membrane were the functional groups of major relevance. In addition, several groups of microbial groups were identified to have different metabolic impacts on leaf litter degradation. *Pseudomonadales* and *Xanthomonadales* showed highest metabolic activity towards the leaf litter. *Burkholderiales* and Bacteroidetes revealed moderate metabolic. In contrast, members belonging to *Actinomycetales* and *Rhizobiales* showed only low degradation activity.

Conclusion
Our study revealed a link of phylogenetic origin and functional information to during nutrient cycling based proteins and provides a deep insight into molecular details of the leaf-litter decomposition process.

P124
Population genomics of the legume plant host specific selection of rhizobial genotypes

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Introduction
Rhizobium leguminosarum bv. viciae (*Rlv*) establish effective symbioses with four legume genera: *Pisum*, *Lens, Lathyrus* and *Vicia*. Classic studies using trap plants provided evidence that, given a choice, specific hosts select specific genotypes of rhizobia which are, apparently, particularly adapted to that host (Mutch & Young, 2004; Louvrier et al, 1996).

Objective
Pooled DNA samples from *Rlv* nodule isolates obtained from different legume plant hosts used as rhizobial traps should allow a test of the hypothesis that different plant hosts select specific subpopulations of rhizobia from the available population present in a given soil.

Materials and Methods
We have applied a Pool-Seq approach (Kofler et al, 2011), to study plant host selection of genotypes from the available rhizobial genomic diversity present in a well-characterized agricultural soil (INRA Bretennieres). Plants of *P. sativum*, *L. culinaris*, *V. sativa* and *V. faba* were employed as traps. We pooled 100 nodule isolates from each host, and the pooled DNAs were sequenced (BGI-Hong Kong; Illumina Hi-Seq 2000, 180 bp PE libraries, 100 bp reads, 12 Mreads). Reads were quality filtered with Trimmomatic, mapped with Bowtie2 using Rlv 3841 as reference genome. Single Nucleotide Polymorphisms (SNPs) were called with VarScan. Results were visualized with SeqMonk and IGV.

Results
Our results confirm, at the genomic level, previous observations regarding plant selection of specific genotypes. We expect that further, ongoing comparative studies on differential Pool-Seq sequences will identify specific gene components of the plant-selected genotypes.

Conclusions
Since rhizobial populations are a minor fraction of the soil microbiota, any attempt to approach the genomic structure of such populations will necessarily require a preliminary enrichment through the use of legume host trap plants, thus potentially introducing a host-mediated bias.

Our work discussed here provides genomic evidence that specific hosts select specific genotypes from the available variability present in the soil.

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P125
Soil microbial community structure and function in relation to water regime changes in the Namib Desert

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Arid systems constitute the most extensive and one of the harshest terrestrial biome on earth. Contrastingly, their microbial community structure and function dynamics remain largely unknown. In this environment, microbial metabolism is strongly affected by water availability (e.g. timing, intensity and frequency of water pulse events) and is expected to vary diurnally in relation with soil moisture levels and temperature. We aimed to evaluate and determine factors driving the dynamics of desert soil microbial community structures and functions using a combination of molecular tools (16S rRNA gene T-RFLP), ecophysiological methods (enzyme assays) and environmental measurements (soil chemical parameters). *In situ* and controlled microcosm experiments were performed to evaluate the impact of (i) soil moisture and temperature variation during diurnal cycles and (ii) distinct water pulses on microbial community structure and functions. In a field study in the Namib Desert, we sampled a single site over a 5 day period, 3 times per day (early morning, midday and night) to reflect diurnal cycles. Soil surface temperature and moisture were recorded during the entire sampling period. In a

microcosm study, we tested the effect of water pulses frequency and intensity on Namib Desert soil microbial communities from 2 soils with different water-regime histories (upland soil or dry riverbed sediment). The microcosms were placed in temperature controlled incubators for a period of 5 weeks, received water pulses mimicking fog, episodic rain or heavy rainfall, and were collected at 8 occasions. Pulses simulating fog and episodic rain generally triggered the most microbial activities in the microcosms compared to heavy rainfall simulation. Contrastingly, when assessed *in situ*, desert microbial community showed highest activity in the middle of the day when soil moisture was reduced and lowest activity during the night when soil moisture increased. These studies underlined the complexity of desert systems and provide fundamental description of microbial community dynamic during water regime variation as well as valuable information on the reaction of arid ecosystems to global climate changes.

P126
Soil environment as a source of new tools for biotransformation and bioremediation

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Soil microbial communities present one of the highest level of bacterial diversity on the Earth. These microorganisms play a major role in the biogeochemical cycles of the biosphere but also in utilization of xenobiotics such as pesticides. Some of pesticides are chemically stable and have tendency to accumulate in food chain, causing harmful effect in human and animals. Next generation sequencing combined with metagenomics creates opportunity to gain insight into highly complex genoms of soil microorganisms and search for new tools and methods for bioremediation. Aim of this work was to examine with metagenomic methods if bacteria present in chemically contaminated soil can be a source of novel enzymes useful for in situ bioremediation. Total DNA was isolated from contaminated soil samples collected during dismantling of infrastructure build for storage of expired pesticides. Soil contamination was analyzed with GC-MS and HPLC-MS. After sequencing with Illumina platform data were assembled with either Velvet, MetaVelvet or CLC Genomics Workbench software. Identification of potential ORF's with MetaGeneMark was followed by data comparison to non-redundant protein database using blastp and annotation of functional domains using HMMER3 and PFAM database. Potential enzymes from atrazine degradation pathway were cloned, overexpressed in E.coli and purified. Enzymatic activity of proteins were tested using colorimetric method with pyridine (Tawfik et al, 1968) followed by GC-MS. Chemical analysis indicated that DDT, HCH or atrazine were the most common pollutants in soil samples. Bioinformatic analysis of NGS data resulted in selection of DNA sequences coding set of different proteins exhibiting significant level of similarity to known enzymes involved in atrazine degradation. The most active enzyme was able to decompose 40% of substrate in 40 minutes, what was confirmed with GC-MS.

Metagenomic analysis of DNA bacteria from pesticide contaminated soil led to identification of new enzymes exhibiting desired activity against atrazine. However effectiveness of those proteins in bioremediation need further investigation.

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P127
Parallel diversity assessment of rhizosphere and endophytic communities

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A need for toxicity reduction or survival of unfavorable conditions are considered as the main drivers of beneficial plant-microbe interactions. These interactions can take place in the rhizosphere, but some microorganisms can enter the plant body though the root cortex and become endophytes. In order to better understand the processes in functioning ecosystems, the endophytic populations should be studied along with the rhizosphere ones. In order to address this topic, we analyze microbial communities associated with natural vegetation of polluted soils. Analyses are conducted through cultivation-based and molecular methods. Cultivation-based analyses revealed that common soil microorganisms can be found not only in soil, but in plant bodies as well. In order to sufficiently compare rhizosphere and endophytic communities, we have implemented high-throughput sequencing of bacterial 16S rRNA and functional genes amplicons prepared from soil or plant metagenomes, respectively. In general, this methodology can be used for comparison of microbial communities in the rhizosphere and endosphere, for better understanding of plant-bacteria interactions and role of bacteria in the cycling of nutrients.

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P128
Soil Microbial Community Structure vs Function: Who's driving?

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The temptation to link microbial community structure to function has been irresistible as the presence of specific bacteria is often equated with specific functions. With high throughput community sequencing approaches, functions inherent to some environments are derived solely from the microbial community structure, which can be based on phylogenetic markers such as 16S rRNA gene sequences. Yet, considerable study of horizontal gene transfer (HGT) lends credence to functional adaptation as different microbial species have been shown to exchange some genetic material. Using a meta-analytical approach with a range of different metagenomic datasets, microbial community structure and function have been compared. One aspect relates to how the taxonomical classification provides information concerning microbial function. Some phyla share functions, but what is the distribution of very common or uncommon functions between species or genera? The

ecological point of view of microbiology may lead to different function, for example the distribution of some species in correlation with others may generate some cooperative work between some bacteria, or the formation of consortia, which have different functions than the whole community. In the case of there's a link between structure and function, a remaining question is "who's the driver?". If the same structure leads to same functions, slight environmental perturbations may not induced changes into the community, but we will show that for the same microorganisms, the function could be different. If there is a link between structure and function, the link might be conserved through DNA, RNA and protein.

P129
Effect of wetland vegetation, water level and fertilization on bacterial and fungal community composition and activity - mesocosm manipulative experiment

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Increased nutrient levels to wetlands lead to greater plant production, with more biomass allocated to aboveground structures (lower root-to-shoot ratios), changed nutrient contents of plant materials (lower C:N and C:P ratios) and, therefore, faster decomposition rates of plant litter. These changes in the growth and stoichiometry of wetland plants can affect the soil microbe community, resulting in a shift in the fungi:bacteria ratio, faster nutrient turnover rates, and increased CO2 respiration. It is extremely difficult to investigate each of these processes in detail under field conditions so to determine their importance to wet grassland functions. Therefore, there is a need for more controlled studies to investigate these processes in detail.

A mesocosm experiment had a full factorial design consisting of 2 moistures, 2 nutrient levels and 2 soil (peat; mineral) treatments, for a total of 8 treatment combinations. During the second year of the experiment we collected soil from all treatment (80 samples), extracted DNA and sequenced the 16SRDNA and ITS1 amplicons to determine bacterial and fungal community composition, respectively. Bacterial community sequencing was performed using Illumina MiSeq platform (Argone national Lab, US), fungal sequencing was performed using Roche GS Junior Sequencer at the Univ. of South Bohemia. We also determined the abundance of bacteria and fungi by qPCR. The activities of basic soil enzyme were determined.

Our results showed that soil type and presence/absence of plant had the strongest effect on microbial community composition while the addition of NPK fertilizer had the weakest effect. The most abundant bacterial phyla were Firmicutes, Verrucomicrobia, Proteobacteria, Actinobacteria and Acidobacteria. Verrucomicrobia dominated mineral soils while Firmicutes dominated the peat soil.

The most abundant Archaeal genus was Methanobacterium which dominated the anaerobic mineral soils without plants. Presence of plant had also the strongest effect on bacterial alpha diversity (Chao1 index) which was two times higher than without plants. Also the potential soil enzyme activities in involved in C, N and P transformation were almost two times higher in presence of plant.

From the results we conclude that the effect of fertilization is strongly masked by the type of soil and presence/absence of plant.

P130
Changes in bacterial and archaeal community composition in mineral soils during long-term ecosystem development

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During long-term ecosystem development, both soil nutrient contents and mineralogical assemblage change, thus, probably affecting microbial community composition. Factors influencing soil microbial community composition also vary with soil depth. Therefore the aim of our study was to investigate the microbial community structure along a soil development gradient with soil depth. For that, we conducted barcoded 454 pyrosequencing on archaeal and bacterial 16S rRNA genes of mainly mineral soil horizon samples at four development stages along the 120,000 year-old Franz Josef chronosequence (New Zealand). We found a compositional shift in microbial community with ongoing ecosystem development in subsoil horizons. Diversity was highest at young to intermediate-aged sites and declined afterwards. Acidobacteria (≥15%), Planctomycetes, Proteobacteria, Chloroflexi and Firmicutes were identified as the most abundant bacterial groups. Planctomycetes and Proteobacteria (especially alpha-Proteobacteria) occurred more frequently at the young and intermediate-aged sites whereas Firmicutes became more abundant at the oldest site. Archaeal sequences were assigned to Eury-, Cren- and Thaumarchaeota and most abundant groups were Terrestrial Group (≥50%), South African Gold Mine Crenarchaeotic Group 1, Thermoprotei, and Thermoplasmata. The Terrestrial Group and Thermoplasmata were most abundant at intermediate-aged sites whereas there was a shift to a higher proportion of Thermoprotei at the oldest site. To determine how physico-chemical and mineralogical soil properties drive changes in community composition and potentially associated effects on nutrient cycling, further investigation of sequencing data and real-time PCR of functional genes is in progress.

P131
Diversity of soil crust prokaryotic microbiota and its response to physical disturbance

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Biological soil crusts (BSCs) provide important ecosystem services in dryland regions that cover over 35% of the Earth´s land mass, including 24% of Europe. They play vital roles in seedling germination, reducing soil erosion and contribute to nitrogen and CO_2 fixation. The primary objective of the BiodivERsA project Soil Crust InterNational is to elaborate biodiversity conservation and sustainable management strategies for BSCs affected by anthropogenic perturbation. Here, the emphasis is on the diversity of the bacterial soil surface communities in relation to bacteria inhabiting the underlying soil layer. 120 samples from Nature Reserve at Gössenheim (Germany) and Gynge Alvar (Sweden) as

well as Hochtor (Austria) and Tabernas basin (Spain) were analyzed by 454 pyrosequencing targeting the V4 hypervariable region of the bacterial 16srRNA gene. For detection of bacteria on terricolous lichens fluorescence *in situ* hybridization and confocal laser scanning microscopy (CLSM) was utilized.The majority of the 305,838 sequences belonged to *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria*. Both BSCs and soil communities were dominated by the class ***Alphaproteobacteria***. The majority of OTUs was rare and unique to either BSCs or bulk soil communities. UniFrac-based PCoA indicated that crust samples grouped into clusters by location. The small number of shared OTUs across BSCs, particularly in the phyla *Proteobacteria* and *Actinobacteria*, suggests a minimal core microbiome. CLSM revealed colonization by bacterial communities in fungal tissues (cortex, rhizohyphae) of *Psora decipiens* and *Toninia* sp. The results indicate that BSCs harbour a microbiota that is distinct from microbes present in underlying bulk soil and that the composition of these communities diverges across large geographic distances. Next, we will investigate the influence of parameters such as precipitation, temperature and soil properties on the diversity of soil-inhabiting bacteria as well as their response to disturbance in replicated field experiments.

P132

Genetic and functional diversity of soil microbial communities associated to grapevine plants and wine quality

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Introduction

Despite the economic importance of vineyards in Italy, the wine sector is facing severe challenges from increased global competition and climate changes. The quality of the grape at harvest has a strong direct impact on final wine quality and the strong relationship between wine composition, aroma, taste and soil properties has been outlined in the "Terroir concept".

However, information on the impact of soil microbial communities on soil functions, grapevine plants and wine quality is still lacking.

Objectives

The aim of this study was to explore the composition and the potential functions of soil microbial communities associated to grapevine plants grown in two soils which showed similar physical, chemical and hydrological properties but which provided a different wine quality.

Materials and Methods

Soils from two sites of the Chianti region in Tuscany (BRO11 and BRO12) cultivated with the grapevine cultivar Sangiovese with contrasting wine quality were examined by means of a structural and functional approach: specifically, GeoChip microarrays, pyrosequencing of 16S rRNA and 18S rRNA genes, enzyme assays and measurements of some soil biological properties, such as microbial biomass C and soil respiration, were carried out.

Results

Enzyme assays and soil biological analyses revealed a higher biological activity in BRO11 as compared to BRO12. The structure of soil microbial communities, assessed using 16S and 18S rRNA gene-targeted pyrosequencing, revealed a higher presence of Actinobacteria in the BRO12 than in the BRO11 soil where, in contrast, the alfa-Proteobacteria are more abundant. GeoChip microarray analyses revealed a consistent difference in genes involved in S cycling, with a significant overrepresentation of sulfur-oxidation genes in BRO11 and increased levels of sulfate reduction genes BRO12. These results are consistent with the high content of sulfates and the abundance of Firmicutes such as *Sulfobacillus thermosulfidooxidans* in the BRO11 soil.

Conclusion

The preliminary results of this work suggested an active role of microbial communities on grape physiology and "Terroir concept". In particular, the biological oxidation of sulphur in BRO11 appeared to be one of the factors which determines the wine quality. However further studies are needed to confirm this indication.

P133

How do warming and grazing affect soil bacterial diversity in the Mongolian steppe?

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Background

Soils are some of the most biologically diverse habitats on the planet, and a significant portion of the bacterial diversity in these habitats is accounted for by bacteria; however, neither the impact of bacterial diversity on an ecosystem, nor the drivers that shape this diversity are well understood. A pressing question is how soil bacterial communities may be shaped by changes in climate and land use. In northern Mongolia, air temperatures have increased by 1.6 °C since 1960, and changes to grazing patterns are occurring due to migration from the steppe to the city and a shift to more sedentary pastoralism.

Question/Methods

To investigate the interactive effects of climate and land-use change on bacterial communities, soil samples were collected over the course of three years (2009-2011) from an experimental site in northern Mongolia, undertaken by the PIRE Mongolia Project. Climate was manipulated using passive open top warming chambers (OTCs), and was crossed with grazing. DNA was extracted from each sample and 16S rRNA V4 PCR amplicons were sequenced using the Illumina platform through the Earth Microbiome Project (www.earthmicrobiome.org). The resultant community profiles were analyzed using multivariate,

non-parametric statistical tests to explore changes in alpha (i.e., species richness within a sample) and beta (i.e., species overlap between samples) diversity resulting from shifts in grazing intensity and temperature.

Conclusions
Alpha diversity was calculated for each sample using the an observed species count and treatment effects of climate and grazing were tested using the Kruskal-Wallis test. Beta diversity (measured with the Unifrac metric) was determined by comparing all possible pairs of samples to obtain a dissimilarity matrix of phylogenetic relatedness and a PERMANOVA was performed to determine significant treatment effects. Alpha and beta diversity differed between years, but not experimental treatments. These results, indicate considerable temporal variation in soil bacterial diversity, which may be the result of climatic variation between years.

P135
Bacterial diversity in the rhizosphere of maize - Scaling potential effects of a genetic modification to biogeographic variables

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The composition of bacterial communities in rhizospheres is affected by properties of the plant roots and of their surrounding soil. Differences detected between plants of different age as well as cultivars suggest that microbial communities are highly responsive to alterations in the composition of root exudates. On the other hand, genetically modified (GM) crops have generally no or only a very small effect on the bacterial diversity in rhizospheres. The objective of this study was to compare the bacterial and archaeal diversity found in the rhizospheres of maize grown in different climatic regions in Europe and to analyze whether a genetic modification would trigger the same level of response, independent of the site of cultivation. This question has practical relevance, since the centralized approval system for GM plants for agricultural use by the European Commission could eventually allow their cultivation in contrasting biogeographical regions across Europe.

Within the EU-funded project AMIGA (*«**A**ssessing and **M**onitoring the **I**mpacts of **G**enetically modified plants on **A**gro-ecosystems»*) we analyze the structural diversity of bacteria colonizing the rhizosphere of the GM maize MON810. Rhizosphere samples of this GM maize and its near-isogenic, non-GM parental cultivar were collected from field trials in Slovakia, Spain and Sweden in 2012 and 2013. Bacterial profiling by T-RFLP analysis of partial 16S rRNA genes revealed significantly different rhizosphere bacterial communities for the three locations and between cultivars in Sweden and Spain, but not in Slovakia for 2012. Furthermore, the abundance (as determined by quantitative real-time PCR) of Bacteria and Archaea differed significantly between the locations and for Bacteria in Sweden and Archaea in Spain as well between cultivars.

The structural diversity of the bacterial communities is currently also assessed by Illumina MiSeq sequencing of PCR amplicons. Thus, we expect to be able to distinguish a core bacterial community of maize from a more locally defined community which responds e.g. to soil properties, agricultural management practices or climatic conditions.

P136
Phages as vectors and indicators of biological information in the Earth's Critical Zone

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Bacteriophages are viruses that infect specific bacteria and thus have a vast influence on their hosts in terms of mortality, community structure and biogeochemical cycles. Although they represent the most abundant biological entities on earth, research has focused mainly on marine ecosystems neglecting terrestrial habitats. Moreover, the implications of viruses for the genetic landscape of the deep subsurface biosphere in terrestrial ecosystems are still almost *terra incognita*.

With this poster we would like to present and discuss our recently started project, which is funded by the DFG Collaborative Research Centre 1076 - AquaDiva. The overall aim of this project is to study (transducing) phages as vectors of the soil bacterial metagenome in the subsurface part of the Earth's Critical Zone. We want in particular to generate an overall inventory of viral communities (morphotype and molecular diversity) in selected field core samples and to investigate the viral functional metagenome using 454 shotgun sequencing. Furthermore we aim at identifying 16S rRNA genes transduced by bacteriophages using 454 pyrosequencing to reveal bacteria participating in transduction-mediated gene transfer in different layers of the Earth's Critical Zone.

P137
Soil particle size fractions harbour microbial communities with different metabolic potentials and activities to degrade phenol

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Most soil microorganisms live in close contact with organo-mineral surfaces. Depending on the particle size fractions (PSF), categorised as clay, silt and sand, surface properties can be very different and consequently may select for structurally different microbial communities. The aim of this study was to explore and compare the capacity of microbial communities of different particle size fractions to degrade the organic pollutant phenol, and to elucidate their response to environmental variables as induced by different long-term fertilisation regimes. For this purpose, soil variants from the Askov Long-Term Experiment (DK), which share the same particle size distribution and pH value but differ in their amount and quality of soil organic matter, were separated by mild ultrasonication, wet-sieving and centrifugation. After DNA extraction from separate PSF, qPCR and TRFLP were conducted to reveal the abundances and to fingerprint the diversity of *Archaea*, *Bacteria* and *Fungi*. To investigate the degradation of phenol, the PSF were mixed with sterile quartz, and isotope-labelled phenol was added, i.e., ^{14}C for mineralisation studies and ^{13}C for stable isotope probing (SIP). The abundance of all three domains increased by 3-4 orders of magnitude from the sand-sized to clay fractions. Independent of the PSF, manured soil variants showed higher microbial abundances. The structural diversity of the bacterial and fungal communities were mainly shaped by the different PSF. Fertilisation practices also affected the diversity of

the microbial communities, especially the *Archaea*. Phenol mineralisation activity correlated with microbial abundances of the PSF with the exception of coarse silt showing the lowest activities. PSF from fertilised variants were more actively degrading the compound. Currently SIP analyses are conducted to reveal the identity of the microorganisms involved in the degradation of this compound.

P138

Variations in the structure and functional activity of soil microbial communities associated with phase of crop rotation and annual rice cycle

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The agricultural productivity and soil fertility are intrinsically associated with the soil microbial communities. Thus, the assessment of possible correlations between microbial community structure and activity, and external factors may contribute to further improve agriculture practices. In the present study, we analysed paddy soils subjected to a rotation of alfalfa (two years) and rice (two years). During the second half of the rotation cycle, *i.e.*, 1st and 2nd year of rice cropping, samples were collected before seeding, at the maximum tillering phase and after harvesting, and were characterized for physicochemical, biochemical and microbiological traits. Variations possibly due to the (i) phase of crop rotation and (ii) phase of annual rice cycle on the bacterial community structure and functional activity of these soils were assessed using multivariate analyses.

The results showed that the phase of crop rotation coincided with variations in the composition and structure of the soil bacterial community. In the 1st year of rice cultivation, the abundance of aerobic heterotrophs was positively correlated with lineages affiliated to *Rhizobiales*, *Rhodospirilales*, *Sphingomonadales*, *Flavobacteriales*, *Sphingobacteriales*, among others. In the 2nd year, the abundance of bacteria affiliated to *Bacteroidales*, *Anaerolineae* and *Chlorobi* was positively correlated with the soil carbon content. Variations on the microbial catabolic activity were in agreement with those observed for the community structure. Changes in microbial activity were also detected along the rice cropping cycle. Proteolytic activity and aerobic and anaerobic heterotrophs were predominant before rice seeding. After rice harvesting, an increase of cultivable diazotrophic microorganisms, suggests an increase in N_2 fixation. These results indicate that polyphasic studies may be an interesting contribution for improving agriculture practices.

P139

High elevation grasslands dominated by *Carex curvula* and *Nardus stricta* select for similar fungal communities in the Alps and in the Carpathians.

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Plants are known to influence soil fungal communities either by species-specific interactions or the quantity/quality of plant litter and root exudates [1-3]. Plants and plant communities' seems to select for specific fungal communities composition at the pot, the landscape and the regional levels [4-7], but the question remains at a biogeographical scale. To assess this question, we have studied fungal communities from the soil underneath the plants *Carex curvula* (natural alpine plant) and *Nardus stricta* (anthropic subalpine plant) from Carpathians and the Alps. The samples (3 replicates/site) from 13 Alpine and Carpathian sites were collected in July 2007; soil DNA extraction, fungal ITS1 amplification were performed as previously described [4-6], and submitted to MiSeq pair-end sequencing. Molecular Operational Taxonomical Units (MOTUs) were obtained and assigned as previously described [5]. Assembly rendered 1 147 767 sequences with a length comprised between 100 and 436 nucleotides. Filtering of ambiguous sequences and singletons rendered 864 865 sequences, representing 28 740 unique sequences and 3691 MOTUs. Nonmetrical MultiDimensional Scaling grouped the fungal communities according to plants communities, rather than geographical origin. Moreover, 41 MOTUs were found to be specific for *N. stricta* and 15 for *C. curvula*. These results provide evidence for plant sorting of fungal species at a biogeographical scale despite contrasting history of post-glacial recolonisation history of mountain flora [8].

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P140

Plant species and soil type affect rhizosphere microbial community composition

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Introduction

Soils differ in their chemical and physical parameters which have an influence on plants growing in these soils, for example, via nutrient availability. Different soil parameters may lead to different microbial communities which also interact with the plant and can influence plant health and crop yield.

Objectives

To understand the complex interactions between the soil type, plant and microbial community a field experiment with a plot system containing three soil types (diluvial sand, DS; alluvial loam, AL; loess loam, LL) and two plant species (lettuce and potato) was performed.

Materials and Methods

Total community DNA from the rhizosphere of the plants was extracted and analyzed by DGGE as well as by pyrosequencing of 16S rRNA genes.

Results

DGGE analysis showed that the rhizosphere microbial community composition of lettuce and potato in the three soil types differed significantly. Amplicon pyro-sequencing of 16S rRNA genes from rhizosphere samples from lettuce and potato revealed that *Proteobacteria, Actinobacteria, Firmicutes, Bacteroide-tes* and *Acidobacteria* were the dominant phyla. The *Proteobacteria* have the highest relative abundance in the three soil types. Within the *Proteobacteria,* the *Alphaproteo-bacteria* were the most prominent class, followed by the *Betaproteo-bacteria* which were enriched in the rhizosphere in lettuce grown in all three soil types. UPGMA based on Pearson correlation showed a clear separation of the lettuce and potato community structure in the rhizosphere. In the potato rhizosphere three sub cluster caused by the soil type could be observed, the LL soil being more similar to the DS than to the AL soil. In the rhizosphere of lettuce two sub clusters were caused by the soil types LL and DS. Two of the four replicates of the AL soil clustered with DS, the other two with LL. The OTU report showed that in the rhizosphere of lettuce and potato different OTUs were enriched. Whereas in the rhizosphere of lettuce for example *Lysobacter sp.*, *Streptomyces scabies*, Sphingobium and *Shinella* were enriched, *Acidovorax*, *Methylibium*, *Burkholderia*, *Azospirillum,* and *Massilia* were enriched in the rhizosphere of potato.

Conclusion

In conclusion, the plant species and the soil type had a strong influence on the bacterial community in the rhizosphere.

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Responses of tundra soil bacterial communities to increased nitrogen availability

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Arctic tundra soils store large quantities of global organic carbon as the decomposition of plant litter and soil organic matter is limited by low temperatures. On the other hand tundra soils are generally N limited which may further limit decomposition. Increased N availability has been, however, also shown to suppress organic matter decomposition which has been linked to a mechanism where microbes in N-poor soils utilize complex organic compounds as nitrogen sources while growing on more labile carbon (nitrogen mining). To elucidate the role of increased N availability in tundra soil, we conducted an incubation experiment and traced the effect of added N on microbial activities, biomass and bacterial community structure. Microbial activities were evaluated by CO_2 production and extracellular enzyme activities; biomass was estimated as microbial-N, PLFA concentrations and bacterial and fungal rRNA gene copy numbers. Bacterial community structure was characterized by Ion Torrent sequencing of 16S rRNA amplicons.

Increased N availability resulted in a decrease in respiration and peptidase activity which was coupled to decreased microbial N and soil PLFA concentrations indicating a decline in the microbial biomass. However, qPCR analysis showed increases in both bacterial 16S rRNA and fungal 18S rRNA copy numbers which may indicate shifts in the communities from oligotrophic (low 16S/18S rRNA gene copy numbers) to copiotrophic (higher number of 16S/18S copies) species in nitrogen amended mesocosms. Ion Torrent sequencing indicated significant shifts in the bacterial community composition after 6 week incubation in nitrogen amended mesocosms compared to the control soils. These included a decrease in the relative abundance of phyla that are linked to oligotrophic environments and low growth rates (*Acidobacteria*, *Verrucomicrobia* and *Planctomycetes*) and an increase in phyla that are linked to more copiotrophic microorganisms (*Gammaproteobacteria*). In addition a decrease in *Deltaproteobacteria* and *Bacteroidetes* and an increase in the relative abundance of *Actinobacteria* was detected in the nitrogen amended mesocosms. These results indicate that nitrogen availability controls strongly the microbial community structure with important consequences on the decomposition of organic carbon in tundra ecosystems.

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Taxonomic and functional profiling of the microbial community from a thermophilic production-scale biogas plant by a metagenome approach

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In principle, anaerobic digestion of biomass for biogas production can be practiced under mesophilic or thermophilic conditions. Thermophilic digestion offers the opportunity to achieve higher methane yields and to treat problematic substrates. However, stability and control of thermophilic processes are demanding. Moreover, very little is known about microbial communities of thermophilic biogas reactors. To further optimize the biogas production process, characterization and estimation of the diversity and genetic potential of microbial communities residing in thermophilic biogas reactors is absolutely essential.

To analyze the composition and functional potential of the microbial community prevailing in a production-scale thermophilic biogas plant, a metagenome approach on the Illumina MiSeq system was applied. Moreover, it was assumed that the method for DNA-extraction from complex fermentation samples greatly affects taxonomic classification results. For this reason, total community DNA was extracted from fermenter samples using five different protocols. Metagenomic DNA was sequenced and analyzed by means of the metagenome analysis platform MGX. All identified phyla of the biogas plant were previously described for other biogas fermentation reactors. Within the domain *Bacteria*, high abundance of the class *Thermoanaerobacterales* and *Halanaerobiales* could be shown, whereas members of the class *Bacilli* occurred less frequently. *Methanomicrobia* dominated within the methanogenic sub-community. Furthermore, detailed comparison of taxonomic profiles revealed that the DNA extraction method greatly affects taxonomic profiling results. Further evaluation of DNA extraction protocols is required to achieve reliable representation of taxa within taxonomic profiles. However, the metagenome approach carried out in this study provided first insights into the composition and functional potential of the microbial community from a thermophilic biogas plant.

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Microbial communities of different dimensional groups of soil aggregates under the extreme agricultural systems

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The patterns of soil microbiome structure may be an universal and very sensitive indicator of soil quality (soil "healh") used for optimization and biologization of agricultural systems. The basic structural and functional unit of the soil is a soil aggregate, which is actually a microcosm of the associative co-existing groups of microorganisms that form characteristic ecological food chains. Recent research demonstrated that the microbial metabolic quotient was lower on the aggregates' surface in comparison to the inner part of the soil structural units. The occurrence and the intensity of soil processes as well as the soil fertility could be conditioned both by the dimension of the aggregate and the system of land use.

The objective was to determine the microbial community structure of chernozem in three different dimensional groups of soil aggregates (less than 0.25 mm (fine), 2-5 mm (middle) and more than 7 mm diameter (coarse)) isolated from samples of the following variations of the stationary field experience: continuous wheat, rest soil and wild soil.

The total DNA was extracted from all of the samples and analysed by quantitative PCR (real-time PCR) and by using GS Junior pyrosequencing ("Roche", Switzerland).

Comparision analysis of the rarefraction curves demonstrated that the microbial community of fine aggregates were characterized by the highest richness in comparison to the middle and coarse aggregates. The bacteria from phylas of *Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, Bacteroidetes, Firmicutes, Gemmatimonadetes* were dominating in microbial associations. The microbial community of fine aggregates of the wild land was characterized by the presence of Archaea domain representatives, which could indicate an anaerobic conditions within these structural units. The biomass of fungi and actinomycetes was higher in fine and middle aggregates of the soil under the continuous wheat (in comparison with samples isolated from wild and rest soils). It could be explained by better aeration due to soil treatment and the presence of crop residues.

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Seasonal dynamics of plant associated bacterial communities in low arctic fell tundra

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We are interested in the structure and function of soil and plant associated bacterial communities in the Arctic. In this study, we addressed seasonal dynamics of endophytic and soil bacterial communities associated with Diapensia lapponica in low arctic fell tundra in northwestern Finland. D. lapponica is an arcto-alpine plant species with circumpolar distribution, and grows on exposed fell tops, ridges and frost boil mounds, which are snow free in the winter. D. lapponica is a cushion-forming evergreen perennial shrub, capable of photosynthesis very early in the arctic spring, and hosts a diverse endophytic bacterial community dominated by Proteobacteria and Actinobacteria.

We sampled plant roots (endosphere), rhizosphere and bulk soils in July (early growth season), September (end of growth season), February (mid-winter) and May (early spring), and used pyrosequencing targeting the V5-V6 regions of 16s rRNA gene to analyze the bacterial community structure in different seasons. The endosphere communities were in all seasons dominated by Proteobacteria and Actinobacteria, whereas the main bacterial classes in the rhizosphere and bulk soils communities were Acidobacteria in subgroups 1,2 and 3 and Proteobacteria.

In the endosphere, we detected clear seasonal dynamics, with significant increase in relative proportions of α-Proteobacteria (in particular OTUs representing Bradyrhizobium and unclassified

Rhizobiales) and Acidobacterial subdivisions 1 and 3, accompanied by decrease in Gammaproteobacteria, in the winter. Increased endorhizal α-diversity and increase in resemblance of root and soil communities in mid-winter suggest root turnover or inactivation of plant defense responses in mid-winter. The phyla level community structures in rhizosphere and bulk soil were stable throughout the seasons. However, the relative abundances of several acidobacterial (OTUs representing subdivision 2 and Granulicella) and proteobacterial (Burkholderia) groups correlated with season.

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Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating wet and dry fermentation

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Biomass fermentationfor biogas production can be practiced under wet and dry fermentation conditions. In contrast to the latter, wet fermentation is characterized by a high liquid and a relatively low total solid concentration. Here the composition and functional potential of a biogas-producing microbial community in an agricultural wet fermentation plant was analyzed by means of a metagenomic approach applying 454-pyrosequencing and compared to corresponding data from a dry fermentation process meeting identical conditions with respect to sample and community DNA preparation, sequencing technology and bioinformatic analyses.

Wet fermentation community DNA metagenome sequencing on the Genome Sequencer FLX system resulted in 1,532,780 reads, an average read length of 397 bp and 593.7 mill. bp of sequence information in total. Taxonomic comparison from wet and dry fermentation revealed microbial profiles with *Bacteria* as the most predominant superkingdom, while *Archaea* were less abundant. In both biogas plants, the bacterial phyla *Firmicutes*, *Bacteroidetes*, *Spirochaetes* and *Proteobacteria* were identified with descending frequencies. Amongst the dominant archael phylum *Euryarchaeota* the *Methanomicrobia* was the most abundant class. 16S rDNA amplicon analyses disclosed differences in the sub-communities comprising methanogenic *Archaea* between the processes. Fragment recruitments of metagenomic reads revealed high relatedness of dominant methanogens within the dry fermentation process to the reference genome of the Archaeon *Methanoculleus bourgenis* MS2^T.

Despite major differences in wet and dry fermenters analyzed in this study, high similarities at least at higher taxonomic ranks suggest that core community taxa perform key functions in biomass decomposition and methane synthesis. Also,the dominance of highly related *Archaea* to the type strain *M. bourgensis* MS2^T in the dry fermentation process suggests response of methanogens to specific fermentation process parameters.

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Changes in soil microbial community structure under influence of leaching compounds from radioactive oily waste: a column experiment

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Oily wastes are reported to be one of the most serious environmental treats. These wastes contain oily components, water and mineral fraction which can include natural radioactive elements. Components of oily wastes in case of their disposal on soil surface can leach into soil and influence on soil biota. Microorganisms are essential component of terrestrial ecosystems. Waste disposal can affect the structure of soil microbial community.

To assess influence of oily waste containing radioactive elements on soil microbial community, the column experiment was performed in this study. Raw oily waste (H) containing 575 g kg^{-1} of total petroleum hydrocarbons (TPH), ^{226}R 4403, ^{232}Th 2848, ^{40}K 1276 Bq kg^{-1}and waste from which oily components were washed out (R) were used.

Waste samples were disposed on the top of the columns10x10x60 cm. Rainfall from the top of the columns was imitated. Columns without any waste on the top were used as control (C). After 30 days, soil of each column was divided into three parts: layers 0-20, 20-40 and 40-60 cm and analyzed.

TPH content in all three layers of H-columns was significantly higher than in C-columns. No differences in TPH content were obtained between soil samples of R and C columns. Activity concentrations of R^{226} and Th^{232} increased in $R_{0\text{-}20}$ layer in 3,5 and 1,6 times, correspondingly. No increase of Ra^{226} and Th^{232} was demonstrated in $R_{20\text{-}40}$ and $R_{40\text{-}60}$ layers of R-column and all layers of H-columns. Level of soil microbial biomass decreased from upper to lower layer in all columns. In H-column inhibition of C_{mic} was observed. On the contrary, respiration activity did not depend on depth. Only in $H_{0\text{-}20}$ significant increase (2,4 times) of respiration activity was demonstrated. Influence on bacterial community structure was estimated using PCR-DGGE method.

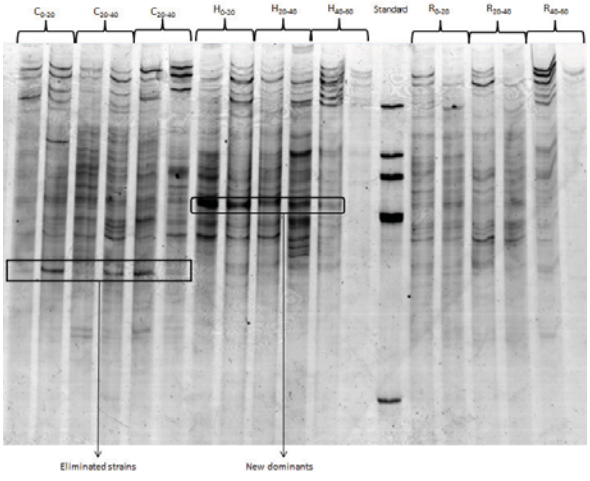

Fig. 1. Structure of bacterial communities of soil layers of C-, H- and R-columns

As shown on Fig. 1, disposal of wastes caused elimination of strains in column H in comparison to column C. New dominant strains were observed in all layers of the H-column. Sample R did not cause visible alteration of soil bacterial community.

Migration of hazardous components of wastes investigated influenced microbiological characteristics of soils. Oily compounds of the waste have more significant influence on microbial communities than radioactive ones.

P147
Detection and characterization of subsurface life in the Iberian Pyritic Belt (IPB).

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From the astrobiological point of view, terrestrial subsurface microbiology is a matter of growing interest. In this context, one of the most fascinating and so far unexplored environments is the Iberian Pyritic Belt, a massive iron-sulfide deposit located in the southwest of Spain. This rather unique environment, that resembles the Mars milieu, sustains a deep subsurface microbial-driven geochemistry that results in oxidation of sulfides in the pyrite to sulfuric acid and acid solubilization of ferric salts to give rise to the extremely acidic Tinto River. Two drilling campaigns were made in subsurface regions where the presence of aquifers could be hosting microbial activity. The rock powder and shards from the central unaltered portion of the core samples obtained were analyzed by Ion chromatography to detect the presence of ions and small organic molecules that indicate metabolic activities. DNA was isolated from these samples and amplified by a MDA reaction using the Phi29 DNA polymerase. Illumina libraries encompassing the V5 and V6 variable regions of bacterial 16S rDNA were generated by PCR and sequenced in the MiSeq platform. Preeliminary results reveal a rich microbial diversity from 100 to 612 meters below ground surface (MBGS), which is dominated mainly by proteobacteria, particularly alpha- and betaproteobacteria. The highest bacterial diversity was found at 420 and 496.65 MBGS, which correlates with small peaks of oxalate, acetate and ammonium. Moreover, a high microbial diversity was also found at 336.5 MBGS, where high concentrations of acetate, Fe^{2+} and Fe^{3+} are present, providing the bacterial community with organic compounds and electron donors and acceptor for supporting metabolic activities. A deep analysis of this unique microbial ecosystem is currently ongoing to further unravel its geobiology and geochemistry.

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The dark side of the metagenomes

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Metagenomic environmental surveys, like the Global Ocean Survey (GOS), generated a huge amount of genetic data and allow performing more holistic approaches to study marine ecosystems. Moreover, metagenomics proofed being valuable in discovering missing links in marine biological processes. Besides expanding our limited view on the diversity of the known protein universe, metagenomics also revealed a large number of genes of *unknown* functions. These can be further classified into I) *known unknowns* like the domains of unknown function (DUF) and II) *unknown unknowns*, putative coding sequences without any hint of potential function. We will present a novel approach to extract valuable information from the co-occurrence of individual protein domains involved in biological processes using Graphical Models. Using an integrative approach, we combine the knowledge of the known protein domain families and 16S ribosomal DNA with the *unknown unknowns* to explore the GOS metagenome. As a result, we were able to reveal new associations in biological processes within known protein families and between known protein families and *unknowns*.

P149
Adaptation of soil microbial community function and structure to chronic metal pollution

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Toxicity of metals released from mine tailings may cause severe damage to ecosystem and human health. A diversity of microorganisms, however, have successfully adapted to such metalliferous sites by a range of resistance mechanisms. In this study, our objective was to describe the indigenous microbial communities existing in mining-impacted soils. To this end, a "total RNA" metatranscriptomic approach was used, retaining both protein encoding as well as ribosomal RNA transcripts. Nine soil samples with low, moderate and high concentrations of cadmium, lead and zinc were investigated (up to 115 g metal/kg soil). Metal concentration and soil pH were found to be the most important factors determining the structure of the soil microbial communities. The most polluted soils showed a higher microbial evenness and taxon richness, compared to those with lower metal concentration. Abundance of Deltaproteobacteria and Betaproteobacteria were positively correlated with metal concentration, while Alphaproteobacteria, Actinobacteria and Acidobacteria were less abundant in contaminated soils.

A number of functional transcripts with consistently and significantly higher expression in contaminated samples were also identified, including genes known to increase resistance to heavy metals. Gene families thought to include such resistance genes

were also increasingly common in more contaminated soils. This study improves our understanding of microbial activity in metal rich environments and can possibly contribute to the development of biological restoration techniques for contaminated sites.

P150
Metagenomics and metatranscriptomics of natural disease suppressive soils

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Previous studies indicated that *Lysobacter* species may play a role in soils which are naturally suppressive to the fungal pathogen *Rhizoctonia solani*. To date, however, the population dynamics of *Lysobacter* species in natural disease suppressive soils and the mechanisms involved in pathogen control remain largely unknown. The overall objectives of this study were i) to determine the abundance of *Lysobacter* species in soils suppressive against *Rhizoctonia solani*, ii) to study their distribution, population dynamics and intraspecific diversity, and iii) to determine if and how they suppress the fungal pathogen *R. solani*. As a first step, we isolated three closely related *Lysobacter* species (*Lysobacter antibioticus*, *Lysobacter capsici* and *Lysobacter gummosus*) from soils naturally suppressive to *R. solani* and tested their antifungal activity. *In vitro* bioassays showed that each of the three *Lysobacter* species inhibited hyphal growth of *Rhizoctonia solani* and of several other fungi, omycetes and bacteria. The abundance of the three *Lysobacter* species in the rhizosphere of sugar beet seedlings grown in soils with different level of disease suppressiveness was determined by a TaqMan detection method. Preliminary results suggest that the *Lysobacter* genus is not more abundant in the rhizosphere of sugar beet seedlings grown in suppressive soils than in conducive soils. To better understand the dynamics and *in situ* activities of *Lysobacter* species and other rhizosphere communities during the transition from a disease conducive to a disease suppressive state, total DNA and RNA were isolated from the rhizosphere of sugar beet and subjected to metagenomic analyses.

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The microbiome of medicinal plants and its potential for biocontrol and promotion of plant growth and quality

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Past medicinal plant research primarily focused on bioactive phytochemicals. Currently, however, focus is shifting due to the recognition that a significant number of phytotherapeutic compounds are actually produced by associated microbes or through interaction with their host plant. Plant-derived medicines have been part of traditional healthcare for thousands of years, and these medicinal plants provide an enormous bioresource of potential use in modern medicine and agriculture, yet their microbiome is mostly unknown. In this study, the microbiome of medicinal plants cultivated on organically managed Egyptian desert farms was investigated and compared to surrounding field and desert soil. The soil microbiome of the desert ecosystem comprised of a high abundance of spore-forming bacteria which were ascertained to be of prime importance for pathogen suppression under arid soil conditions. A clear plant-specific selection of the associated microbes was observed, whereas native desert antagonists were enriched in all investigated plant roots. The anthropogenically influenced ecosystem exhibited a higher microbial diversity and better ecosystem function for plant health in comparison to the natural desert soil. Conversely, several extremophilic bacterial groups decreased or completely disappeared from soil after agricultural use. The diazotrophic community was specific for each medicinal plant as well, indicating that plant species are important drivers for functional diversity. The fungal microbiome was characterised by the presence of potential pathogens, and different desert habitats were screened for antagonists adapted to the unique conditions of desert farming able to biologically control them. In a hierarchical evaluation, three promising candidates were selected for field *ad planta* evaluation in comparison to three allochthonous antagonists. The priming of chamomile seedlings with the autochthonous strains not only showed a stabilising effect on plant performance, but indigenous Bacillales strains were also able to elevate the plants' flavonoid production. These results demonstrate that a targeted bacterial treatment could influence the metabolic activity of the plant, and therefore represent one of many links between the plant-associated microbiome and the plant metabolome.

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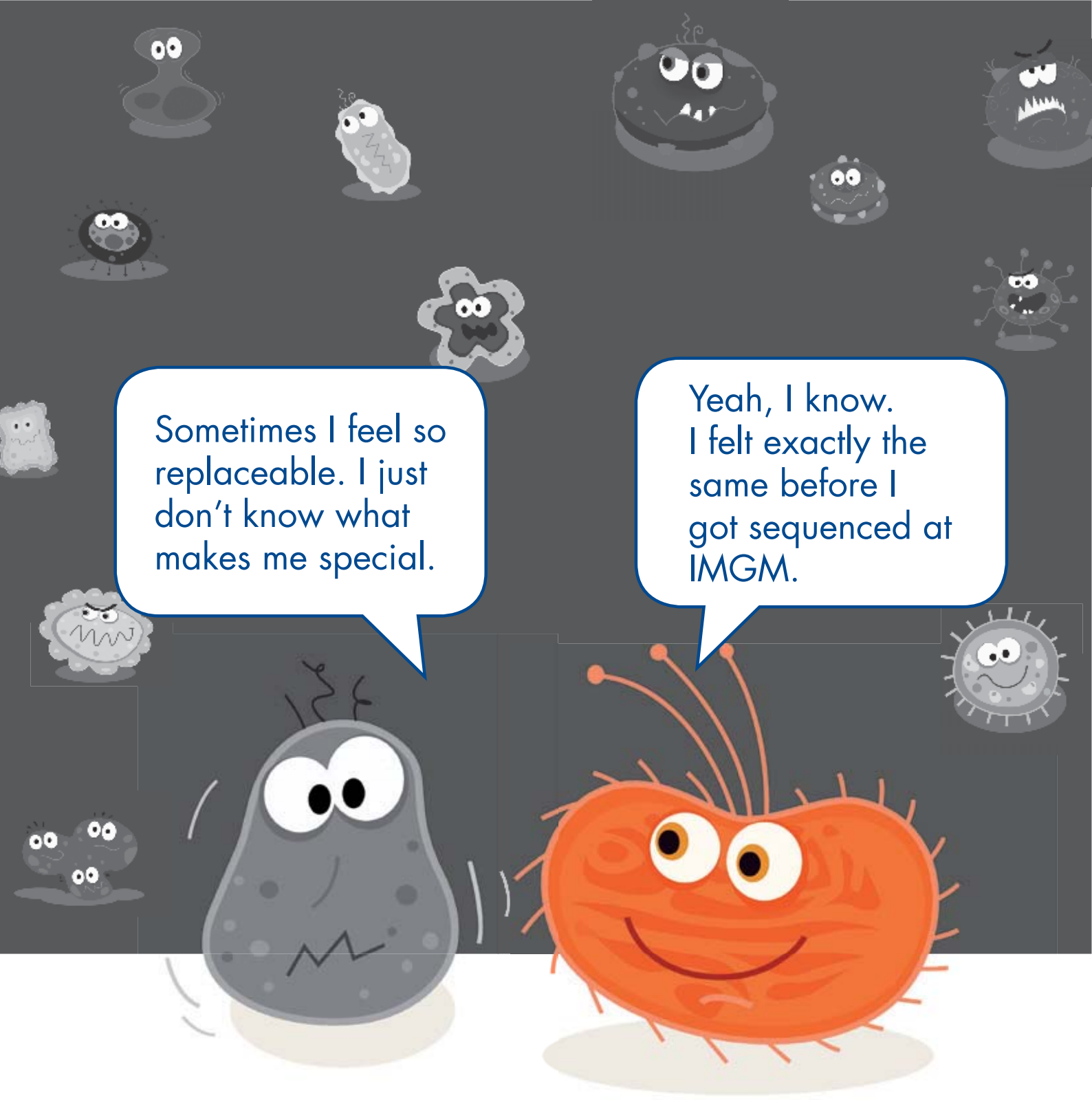

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