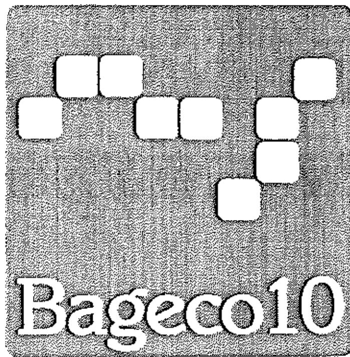

BAGECO 10

Bacterial Genetics and Ecology – Coexisting on a Changing Planet

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Program & Abstract

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P160

EVOLUTION OF GENETIC DEGRADATION POTENTIAL OF PESTICIDE-DEGRADING BACTERIAL COMMUNITIES

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Several bacterial isolates are known to metabolize herbicides belonging to *s*-triazines family among which the herbicide atrazine known to world widely contaminate soil and water resources. The initial hydrolytic dechlorination of atrazine to hydroxyatrazine [2-(*N*-ethylamino)- 4-hydroxy-6-(*N*-isopropylamino)-1,3,5-triazine] is catalyzed by either *AtzA* or *TrzN*. These two enzymes belong to the amidohydrolase superfamily and shared only weak homologies (e.g. *TrzN* from *Arthrobacter aurescens* TC1 shows 26% amino acid sequence identity to *AtzA* from *Pseudomonas* sp. strain ADP). Although *atzA* was the first gene described, *trzN* seems nowadays to be ubiquist in atrazine-degrading microorganisms isolated from geographically different areas. The dechlorination of atrazine is an illustration of functional redundancy where two divergent genes code for two enzymes sharing only low identities but catalyzing similar reactions.
In this context, the aim our study is to study the competition between *TrzN* and *AtzA* from an ecological and a biochemical point of view using a molecular-based approach. To do so, the upper atrazine-degrading pathway (either *trzN, atzBC* or *atzABC*) transforming atrazine to cyanuric acid, was built *in vitro* by a PCR-based approach. Each construction was then subcloned in a surexpression vector and used for transforming *E. coli* competent cells. The ability of either *trzN, atzBC* or *atzABC* genetically modified bacterial strains to degrade atrazine and other s-triazines (simazine, terbuthylazine, atraton,...) were assessed. In addition, a competition experiment was performed to estimate the performance of each pathway. This ecological approach was completed by a biochemical data set aiming at describing the specific activity of each constructed pathways.

MICROBIAL-BIOGEOGRAPHY AT THE SCALE OF FRANCE BY THE USE OF MOLECULAR TOOLS APPLIED TO THE FRENCH SOIL QUALITY MONITORING NETWORK (RMQS).

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Although microorganisms are the most ubiquitous, diverse and abundant living organisms on earth and despite of their key role in a wide range of biogeochemical cycles, few studies (in comparison with macroorganisms) have investigated the distribution of soil microbial community diversity at a scale broader than agricultural fields or forest sites. As a result, the determinism of microbial diversification and the distribution patterns of microbial diversity from small to large scale are poorly documented and understood. As regards to the recent developments of automated molecular tools, such a lack cannot be totally explained by technological limitations, but more by the high difficulty to build up and manage an adequate sampling strategy integrating large spatial scales (landscape, region, territory...) and representative of the environmental variations. In this context, we have investigated the characterisation of the indigenous bacterial communities from soils sampled at a broad scale. This characterisation relied on analyses of density, diversity and genetic structure of bacterial communities by using molecular tools directly on DNA extracted from the soil library of RMQS ("Réseau de Mesures de la Qualité des Sols" = French soil quality monitoring network) which cross-rules all the French territory with about 2200 soils sampled. Physico-chemical characteristics, climatic factors, floristic composition and land use of these soils were also recorded. Specific statistical tools were developed to confront microbiological and pedo-climatic data to the geographic distances in order to i) to characterise the bio-geographical patterning of soil bacterial diversity in French soils, ii) ti deduce the contribution of edaphic, climatic and land use factors on soil microbial diversity and composition, and iii) to validate and compare the taxa-area relationship for bacteria and fungi.

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PLATFORM GENOSOL: A NEW TOOL FOR CONSERVING AND EXPLORING SOIL MICROBIAL DIVERSITY

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Soils are the principal reservoirs of microbial diversity and represent a core component of terrestrial ecosystems. There is an increasing demand for assessing the impact of agricultural and industrial practices on the environment at large scales in a context of global change. To address this demand taxonomic and functional diversity of soil microbial communities, and their stability over time, need to be characterized to better predict soil quality. Recent methodological progresses have led to the development and automation of molecular biological tools (based on the extraction and characterization of nucleic acids), which now allow their application to large scale samplings making possible the production of a reliable reference system for the characterization and interpretation of the soil microbial diversity.

In this context, the Platform « GenoSol » was created in 2008 by the Research Unit of INRA (National Institute for Agronomic Research) « Microbiology of the Soil and Environment » in Dijon (France). The aim of this platform is to provide an appropriate logistic structure for the acquisition, storage, and characterization of soil genetic resources obtained by extensive sampling (several hundred to several thousand soils), on very large space and/or time scales (national soil survey, long term experimental site …), and to make these resources readily available for the whole scientific community and policy makers. The ultimate goal is to produce a reliable reference system based on molecular characterization (taxonomic and functional features) of the soil microbial communities that provides scientific interpretations of the analyses from large scales of time and space sampling. The platform also aims at building up and storing for long term periods a library of soil genetic resources that is made available to national and international scientific communities.

The aim of this poster is to present the technical and logistical tools, the conservatory of soil biodiversity and the scientific objectives as well as the mode of functioning and current research programmes developed in the GenoSol platform (http://www.dijon.inra.fr/plateforme_genosol).

STRUCTURE, DIVERSITY AND DENSITY OF THE PROTOCATECHUATE-DEGRADING BACTERIAL COMMUNITY - IMPACT OF LONG-TERM ORGANIC AMENDMENTS

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Soil systems are considered as carbon sink where aromatic compounds are of crucial interest because of their persistence. The emergence of the need to provide tools for studying bacterial communities ecology and dynamic in link with the biodegradation of these aromatic compounds, led us to use *pcaH* as a molecular marker of the protocatechuate (*pca*) degrading bacterial community. We thus develop a PCR primer targeting a fragment of *pcaH* gene coding for one subunit of the protocatechuate 3,4-dioxygenase, catalysing the ring fission of aromatics. We here, validate the use of this molecular tool by assessing the impact of organic amendment (chemical fertilisation, farmyard manure and sewage sludge), used to fertilise agricultural soils, on the structure, diversity and density of the protocatechuate-degrading bacterial community. Long term amendment (19 years) was performed, on an agricultural soil cropped with maize, as follows: Unamended (U: mineral fertilisation N, P, K); Farmyard Manure (FM: 10 t/ha/yr); Sewage Sludge (SS10: 10 t/ha/yr and SS100: 100 t/ha/2yrs).
The structure assessed by PCR-RFLP fingerprinting showed that *pca* community was importantly impacted in SS100 soil and a slight impact was observed for FM and SS10. The distribution of *pcaH* RFLP types also showed an impact of the amendment on *pca* community structure.
The diversity was studied by Shannon-Weiner and Simpson indexes calculation and by rarfaction curves, both led to the same conclusions that *pca* community was impacted by soil sewage sludge amendment causing an increase of the diversity in SS10 and a decrease in SS100, in comparison with U.
Eventually, the density assessed by real-time PCR quantification of *pcaH* sequences was shown to be significantly higher in SS100 in comparison with U, FM and SS10. To sum up, organic amendments impacted the structure-diversity and density of *pca* bacterial community and huge amounts of organic input (SS100) accentuated the phenomenon.
We therefore validate the use of *pcaH* as an ecological molecular marker of the bacterial community involved in the degradation of aromatic compounds.

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CHARACTERIZATION OF A BACTERIAL CONSORTIUM MINERALIZING ISOPROTURON (IPU)

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A bacterial consortium, isolated from a French agricultural soil, was shown to rapidly mineralize the phenylurea herbicide isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea). Study of the mineralization kinetics by ultra performance liquid chromatography (UPLC) had shown the transitory accumulation and degradation of the three known isoproturon metabolites 3-(4-isopropylphenyl)-1-methylurea, 3-(4-isopropylphenyl)-urea, and 4-isopropylaniline, thus indicating a metabolic pathway initiated by two successive N-demethylations, followed by cleavage of the urea side chain. This bacterial consortium did not degrade other phenylurea hebicides like diuron, linuron, monolinuron and chlorotoluron suggesting the existence of a pathway specific for IPU. Six bacterial strains were isolated from the consortium by successive inoculations on mineral salt medium but none of them was able to degrade isoproturon in isolated form. Based on partial sequencing of the 16S rDNA sequence, the isolated bacterial strains were found to be belonging to genera *Ancylobacter, Pseudomonas, Stenotrophomonas, Methylobacterium, Variovorax and Agrobacterium.* A BAC genomic library was established and is currently under functional screening searching for BAC clone harbouring IPU-degrading capability. Positive BAC clone will then be fully sequenced for identifying gene coding for the enzyme responsible for IPU transformation which up to now remains unknown.

OR31

BACTERIAL AND FUNGAL MCPA DEGRADATION IN THE DETRITUSPHERE

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The detritusphere is characterized by transport of low molecular weight organic substances from the litter into the soil. A small-scale study was performed to clarify whether these organic compounds stimulate bacterial and/or fungal degradation of 2-methyl-4-chlorophenoxyacetic acid (MCPA). We established a microcosm experiment including the following treatments: (1) MCPA, no litter, (2) MCPA, litter, (3) no MCPA, no litter, and (4) no MCPA, litter. MCPA mineralization was measured by radiorespirometry using ^{14}C-ring labelled MCPA. Micro-scale sampling of soils (0 – 10 mm) gave us the opportunity to quantify the MCPA degrading bacterial community using quantitative PCR targeting on *tfd*A and *tfdA*-like sequences coding alpha-ketoglutarate dioxygenase . Furthermore, indicators of fungal biomass and activity (ergosterol, PLFA and peroxidase activity) as well as microbial biomass and DOC were analysed. Litter stimulated the release of $^{14}CO_2$ in stratified microcosms during an incubation period of 20 days at 20°C. Without litter, the amendment of MCPA increased only the abundance of MCPA bacterial degraders using the *tfdA* encoded pathway. Addition of litter stimulated both the MCPA bacterial degraders as well as the whole fungal community. It is hypothesized that bacteria are responsible for MCPA degradation using the alpha-ketoglutarate dioxygenase pathway, whereas fungi decompose MCPA mainly by unspecific enzymes that are released after litter addition. Further studies will quantify the bacterial and fungal carbon incorporation into PLFA using a SIP technique. Our results will be used to improve the modelling of small-scale microbial growth, degradation and transport of MCPA near the soil-litter interface based on measured densities of functional genes and physico-chemical properties of the chemicals under study.

P161

ROLE OF ANTIBIOTIC-PRODUCING PSEUDOMONADS IN NATURAL SOIL SUPPRESSIVENESS TO FUSARIUM WILT

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Natural disease suppressive soils provide an untapped resource for the discovery of novel beneficial microorganisms and traits. For most suppressive soils, however, the consortia of microorganisms and mechanisms involved in pathogen control are unknown. To date, soil suppressiveness to Fusarium wilt disease has been ascribed to carbon and iron competition between pathogenic *Fusarium oxysporum* and resident non-pathogenic *F. oxysporum* and fluorescent pseudomonads.
In this study, the role of bacterial antibiosis in Fusarium wilt suppressiveness was assessed by comparing the densities, diversity and activity of fluorescent *Pseudomonas* species producing 2,4-diacetylphloroglucinol (*phl*D+) or phenazine (*phz*C+) antibiotics.
Densities of *phl*D+ and *phz*C+ pseudomonads have been evaluated by colony hybridization followed by PCR. Genotypic diversity of antibiotic-producers has been characterized by analysis of the polymorphism of *phl*D and *phz*C and by BOX-PCR fingerprinting for phenazine producers. Biocontrol of Fusarium wilt of flax by of *phl*D+ and *phz*C+ strains representative of the genotypes described has been evaluated in the presence and absence of a non-pathogenic *F. oxysporum* strain Fo47.
The frequencies of *Phl*D+ populations were similar in the suppressive and conducive soils but their genotypic diversity differed significantly. However, *phl*D genotypes from the two soils were equally effective in suppressing Fusarium wilt, either alone or in combination with non-pathogenic *F. oxysporum* strain Fo47. A mutant deficient in 2,4-diacetylphloroglucinol production provided a similar level of control as its parental strain suggesting that this antibiotic might not play a major role. In contrast, *phz*C+ pseudomonads were only detected in the suppressive soil. Representative *phz*C+ isolates of five distinct genotypes did not suppress Fusarium wilt on their own, but acted synergistically in combination with strain Fo47. This increased level of disease suppression was ascribed to phenazine production as the phenazine-deficient mutant was not effective. These results indicate, for the first time, that antibiosis via redox-active phenazines contributes to the natural soil suppressiveness to Fusarium wilt disease and may act in synergy with carbon competition by resident non-pathogenic *F. oxysporum*.

P202

DYNAMICS OF SOIL BACTERIAL POPULATIONS INVOLVED IN DEGRADATION OF ^{13}C-LABELLED WHEAT, RAPE AND ALFALFA RESIDUE AS ESTIMATED BY DNA-SIP TECHNIQUE

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In regard to the importance of Soil Organic Matters (SOM) for many of the soil properties plant productivity depends upon, the preservation and the improvement of SOM content is of central importance for the maintenance of agrosystems productivity. In this context, soil amendment with plant residues is a common agricultural practice to compensate the mineralization of SOM consecutive to arable farming. However, a mismanagement of those organic inputs can also induce negative effects for environment quality in terms of eutrophisation and/or greenhouse gaz emission.
Accumulation of SOM depends greatly from the activity of soil heterotrophic microorganisms responsible for the transformation of crop residues to complex and stabilized organic compounds. In spite of its major role in organic matter degradation processes, the microbial component involved is still poorly documented, particularly in terms of populations and functions. In this context, progresses have to be made to elucidate the interactions between the diversity of soil microorganisms and the dynamics of SOM, which should constitute a decisive step forward the improvement of organic inputs management.
We performed a microcosms experiment in order to, (i) characterize the diversity and the dynamics of the bacterial communities actively involved in the degradation of crop residues, (ii) evaluate the influence of the biochemical quality of the crop residues on the diversity of the degrading microorganisms, and (iii) evaluate the link between the dynamics of the diversity of microbial communities and the fate of crop residues added to soil in terms of mineralisation *versus* storage.
Wheat, rape and alfalfa residues labelled at more than 90% ^{13}C were incorporated into soil microcosms and incubation was conducted over a 120-day period. The dynamics and the diversity of bacterial populations actively assimilating C derived from each crop residues were assessed over the time course of the experiment using the (DNA-SIP (Stable isotope Probing) approaches. In parallel, $^{12}CO_2$ and $^{13}CO_2$ fluxes were monitored in each condition to assess the mineralization of each residue added to soil and to evaluate the impact of crop residues incorporation on SOM content through the calculation of the "priming effect" process induced.

P270

ARBUSCULAR MYCORRHIZAE INFLUENCE THE OCCURRENCE OF SOIL FLUORESCENT PSEUDOMONADS HARBOURING TYPE III SECRETION SYSTEMS

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Type III secretion systems (T3SS) of pathogenic and symbiotic Gram negative bacteria allow direct interactions with eukaryotic cells. Saprophytic fluorescent *Pseudomonas* spp. harbouring T3SS genes ($T3SS^+$) have been more frequently encountered in the rhizosphere than in bulk soil, which suggests a possible interaction between these bacteria and rhizospheric eukaryotes.
Our objectives were to compare, (i) the effect of mycorrhizal and of non-mycorrhizal roots of *Medicago truncatula* on the occurrence and diversity of $T3SS^+$ fluorescent *Pseudomonas* spp. and (ii) the diversity of $T3SS^+$ and $T3SS^-$ bacterial genotypes.
Fluorescent pseudomonads from (i) the rhizosphere of mycorrhizal roots of *M. truncatula* Jemalong J5 (wild-type, Myc+ Nod+) and TRV48 (mutant Myc+ Nod-), (ii) the rhizosphere of non-mycorrhizal roots of TRV45 (mutant Myc- Nod-), and (iii) the corresponding bulk soil were isolated.
Pseudomonas spp. strains harbouring T3SS genes were identified by PCR targeting a conserved *hrc*RST DNA fragment. Genotypic diversity of fluorescent pseudomonads has been described by a whole cell rep-PCR fingerprinting method (BOX-PCR) and 16S rRNA sequencing. For $T3SS^+$ strains the polymorphism of *hrc*RST has been assessed by PCR-RFLP and sequencing.
The occurrence of $T3SS^+$ fluorescent pseudomonads was significantly higher in the rhizosphere of mycorrhizal (J5 and TRV48) than in that of non-mycorrhizal roots (TRV45) or in the bulk soil. Four distinct *hrc*RST genotypes were described, two of which were specifically described for strains associated with mycorrhizal roots. Compared to *hrc*RST negative fluorescent pseudomonads, $T3SS^+$ strains displayed specific BOX-PCR genotypes that were all ascribed to the phylogenetic group of *P. fluorescens* on the basis of 16S rRNA gene identity.
Mycorrhizal roots of *Medicago truncatula* clearly influence the population structure of root associated fluorescent pseudomonads in a different way than non-mycorrhizal roots. The occurrence of particular genotypes of fluorescent *Pseudomonas* spp. harbouring T3SS genes was highly increased in the rhizosphere of mycorrhizal roots suggesting that T3SS might be implicated in cellular interactions between fluorescent pseudomonads and arbuscular mycorrhizae.

P55

CHARACTERIZATION OF DENITRIFICATION GENE CLUSTERS OF SOIL BACTERIA VIA A METAGENOMIC APPROACH

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Screening of metagenomic DNA libraries to detect clones whose inserts contain genes of interest is one of the technical challenges related to the development of the metagenomic approach.

A technique was developed in which the 77 000 clones of a metagenomic library are spotted on high-density membranes and hybridized with a probe solution consisting of a mixture of oligonucleotides complementary to 14 different genes. The pool of targeted genes included those associated with functions as wide as denitrification, antibiotic resistance, and dehalogenation.

After hybridization, 134 positive clones were detected out of the 77 000 tested, thus providing a drastic selection process. Positive clone DNA was pooled and pyrosequenced, and sequences compared (BLAST) to those obtained by 454FLX pyrosequencing of the original extracted metagenomic DNA.

In the case of the denitrification genes, contig assembly with bioinformatics tools produced 5 contigs containing *nirS*, 4 contigs containing *nirK*, 2 contigs containing *nosZ* and 1 contig containing both *nirK* and *nosZ*. This study demonstrates the potential of metagenomic approaches to characterize functional genes present in small populations (<5%) of the soil microbial community.

P80

GENEFISHING: AN ALTERNATE METAGENOMIC APPROACH FOR CAPTURING TARGETED BACTERIAL DIVERSITY IN AN ENGINEERED RECIPIENT *E. COLI* STRAIN

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The metagenomic approach, defined as the direct recovery and cloning of bacterial DNA from the environment in domesticated bacterial hosts has been widely used to study bacterial populations and their functional genes in numerous environments. The advantage of this approach over conventional culture based techniques is that it encompasses a wider range of bacteria by bypassing the bias of uncultivability of more than 99% of the bacteria in soil. However, in complex and rich environments such as soils, the huge level of bacterial diversity requires construction, handling and screening of several million clones in order to cover a significant proportion of bacterial genes in the indigenous community. These methods are time and money consuming, and require access to specialized robots that are unavailable to most microbial ecology laboratories. Our objectives were to develop an alternative metagenomic approach in which only bacterial recombinant clones harbouring inserts with sequence based selected genes could develop on growth media. This positive screening technology, called "Genefish" is based on homeologous recombination to extract specific genes from the metagenome into the specifically engineered recipient *E. coli* strain. The key characteristic of this approach is the use of two inducible lethal genes to kill non recombinant bacteria. We will present molecular details of this "Genefish" recipient *E. coli* strain and our first results of its *in vitro* and *in situ* use to extract denitrification related genes from the soil metagenome.

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SUMMER DROUGHT AND FERTILIZER APPLICATIONS AFFECT DENSITIES OF DENITRIFYING BACTERIA IN A PASTURE SOIL

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An important part of the land area in the altitude regions of central Europe is formed by pastures. Pastures often receive high amounts of nitrogen fertilizers, which are known to increase the emission of nitrous oxide (N_2O) immediately after application. Most of the N_2O emitted from soil is expected to derive from the denitrification process when soil moisture is high and sufficient substrate (nitrate) and easily available carbon sources (DOC) are present. Under a changing climate, expected alterations in temporal patterns of rainfall and a postulated reduction of precipitation during summer will influence soil moisture as well as plant performance and therefore microbial activity in soils. This probably affects also the denitrifier community in soil which is responsible for a substantial part of N_2O production as well as for N_2O consumption (further reduction of N_2O to N_2). The aim of the present study was to investigate the effects of summer drought (reduction of precipitation) and different fertilizer types (mineral N, sheep urine and farmyard manure, FYM) on N_2O emissions and densities of soil denitrifying bacteria of a pasture in the Swiss Alps. The experimental plots were fertilized with 300 kg N ha^{-1} a^{-1}. Half of the plots were covered with roofs to reduce precipitation during the summer 2007 and 2008. Annual precipitation (2007: 1800 mm) was reduced by this treatment by ~25%. N_2O production rates were measured weekly and daily after fertilization. Soil temperature and moisture were monitored in the field. Densities of total bacteria and denitrifiers were monitored before and three weeks after each fertilization in 2007 and 2008 by quantifying the 16S rRNA and the *narG*, *napA*, *nirK*, *nirS* and *nosZ* denitrification genes by real-time PCR.
The N_2O emission was strongly reduced by the drought treatment, whereas FYM and especially urine fertilization increased N_2O efflux from soil in the no-roof controls plots. First results from Sept. 2007 showed that the densities of total bacteria and denitrifiers were significantly reduced by drought. In contrast, inorganic-N fertilization slightly increased the density of total bacteria while both the density of total bacteria and denitrifiers was stimulated by organic N-fertilizers. The results of the present experiment indicate that denitrifying bacteria in soil can be heavily affected by drought and fertilization, leading to modified potentials of pasture soils for greenhouse gas emissions.

P205

SPATIAL PATTERNS OF BACTERIA SHOW THAT MEMBERS OF HIGHER TAXA SHARE ECOLOGICAL CHARACTERISTICS

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Whether bacteria display spatial patterns of distribution and at which level of taxonomic organisation such patterns can be observed are central questions in microbial ecology. To investigate the spatial distribution of bacterial groups at high taxonomical levels, we quantified the abundance of eight bacterial taxa at the phylum or class levels in a pasture by using quantitative PCR. Geostatistical modelling was used to analyse the spatial patterns of the taxa distributions. The distributions of the relative abundance of most taxa displayed strong spatial patterns at the field scale (2 to 37 m). These spatial patterns were taxon-specific and correlated to soil properties, which indicates that members of a bacterial clade defined at high taxonomical levels shared specific ecological traits in the pasture. Ecologically meaningful assemblages of bacteria at the phylum or class level in the environment provides evidence that deep branching patterns of the 16S rRNA bacterial tree are actually mirrored in nature.

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MAPPING SPATIAL PATTERNS OF DENITRIFIERS FOR BRIDGING COMMUNITY ECOLOGY AND MICROBIAL PROCESSES ALONG ENVIRONMENTAL GRADIENTS

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There is ample evidence that microbial processes can exhibit large variations in activity on a field scale. However, very little is known about the spatial distribution of the microbial communities mediating these processes. Here we used geostatistical modeling to explore spatial patterns of size and activity of the denitrifying community, a functional guild involved in N-cycling, in a grassland field subjected to different cattle grazing regimes. We observed a nonrandom distribution pattern of the size of the denitrifier community estimated by quantification of the denitrification genes copy numbers with a macro-scale spatial dependence (6 to 16 m) and mapped the distribution of this functional guild in the field. The spatial patterns of soil properties, which were strongly affected by presence of cattle, imposed significant control on potential denitrification activity, potential N_2O production and relative abundance of some denitrification genes but not on the size of the denitrifier community. Absolute abundance of most denitrification genes was not correlated with the distribution patterns of potential denitrification activity or potential N_2O production. However, the relative abundance of bacteria possessing the *nosZ* gene encoding the N_2O reductase in the total bacterial community was a strong predictor of the $N_2O/(N_2+N_2O)$ ratio, which provides evidence for a relationship between bacterial community composition based on the relative abundance of denitrifiers in the total bacterial community and ecosystem processes. More generally, the presented geostatistical approach allows integrated mapping of microbial communities, and hence can facilitate our understanding of relationships between the ecology of microbial communities and microbial processes along environmental gradients.

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MICROBIAL COMMUNITIES OF THE KANCHENJUNGA MOUNTAINS, NEPAL HIMALAYA: FACTORS AFFECTING ABUNDANCE, CULTURABILITY AND ACTIVITY OF THE HIGH-ALTITUDE COLD-TOLERANT MICROBES

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The complex of the Himalaya range is a unique environment at high elevation where steep environmental gradients exist as a result of land uplift. Soils at elevations ranging from 5000 m to 6000 m have recently transitioned from snow or ice covered permafrost to exposed, non-continuous permafrost causing more extreme conditions for the microbial community in terms of desiccation and daily freeze-thaw cycles. The direct counts revealed 10^7 to 10^8 cells / g of dry soil. Comparative 4°C viable counts on various media revealed the psychro-tolerant and fast- growing character of the culturable portion of the microbial community that positively correlated to 4°C respiration ($r = 0.78-0.88$; $P < 0.05$). Redundancy analysis indicated that total soil nitrogen and pH were successful in explaining the variance in abundance, low-temperature culturability and activity (72% and 14%, $P = 0.002$ and $P = 0.05$, respectively), whereas the effects of altitude or presence of vegetation were not significant. Freeze-thaw cycle experiments reproducing 50 daily in-situ temperature cycles resulted in up to five-fold decrease in culturability and a 10% decrease in respiration, values much lower than for temperate soil microbial communities in the same experiments. The data suggest the Himalayan soil habitats harbor abundant microbial populations whose culturable members have physiological traits and phylogenetic affiliations consistent with the non-continuous permafrost conditions of the high altitude Himalayan soils.