"Understanding the *functioning* of microbes and microbial communities in their natural habitat: are we shedding light in the black box?"

Venue and dates: "The power of the small". 14th International Symposium on Microbial Ecology (ISME), 19-24 august 2012, Copenhagen, Denmark.

Proposer and organiser: Paul L.E. Bodelier (PL, MECOMECON).

Summary

The international symposium for Microbial Ecology is the largest scientific event in the field of microbial ecology, bringing together more than 2000 scientist on a biannual basis. For microbial ecologists this is the ideal platform for dissemination and interaction with their discipline at the meeting held in Copenhagen, August 19-24. The four microbial oriented CRP's (FREDI, MOCA, DEEP_C, MECOMECON) within EuroEFFG presented their research in light of the general theme of the activity. The four CRP's represent leading groups covering aquatic (fresh and marine) as well as terrestrial habitats and investigate microbial communities from the single cell to the community level. Hence, the line up of speakers during the oral session guaranteed a comprehensive view on the question whether we are advancing in our understanding of the functioning of microbes in their natural habitats. There was a general consensus among the speakers that genomics has advanced our view on the functional and genetic potential of microbial communities but the true advancement in the field has to come from combining genomic techniques with experimental manipulations and ecophysiological studies of intact microbes and microbial communities, assessed at relevant scales of functioning and interaction. To open up the black box, traditional microbiological investigations are more needed than ever and we need to balance the efforts and resources put into large sequencing projects and traditional microbiological experimentation. Hence, question and hypotheses driven research should be in balance with technology driven genomic studies. Platforms like EuroEEFG, where the advancement of fundamental science through cooperation and networking is the primary goal are ideal for this purpose. The 2 day poster session enabled the PhD students and post-docs to present their work within EuroEEFG and to expand their scientific networks. The interaction with the best scientist in their field is a critically important experience which substantially contributes to the training and career building aspect of the programme. In summary it can only be concluded that this event disseminated EuroEEFG in the most efficient way and facilitated the networking and exchange of four CRP's setting research agendas for future projects.

Final programme of the event

Oral contributed session 35 (Thursday August 23, 13.30-15.30)

- [1]***13.30-13.45: Introduction to the session and to the EuroEEFG programme***. Dr. Paul L.E. Bodelier **(PL; MECOMECON)**, Netherlands Institute of Ecology, Wageningen, the Netherlands. Department of Microbial Ecology.
- ***13.45-14.00:*** *Exploring* ***trophic interactions in the microbial web of the deep-sea".*** Prof. Dr. Thorsten Stoeck **(PI; DEEP_C)**. University of Kaiserslautern, Germany. Department of Biology.
- ***14.00-14.15: "From communities to single cells: finding and defining functional coherence in the lake microbiome".*** Prof. Dr. Stefan Bertilsson **(AP; FREDI**), University of Uppsala, Sweden, Department of Ecology and genetics.

- ***14.15-14.30:*** **What can we learn on ecology of the uncultivated acI lineage of freshwater *Actinobacteria* from genomic analyses?** Hans-Peter Grossart **(PI; FREDI)**. Leibniz Institute for Freshwater Ecology and Inland Fisheries, Berlin. Department of Limnology of Stratified lakes.
- ***14.30-14.45: Living together apart: soil metagenomics and microbially relevant scales". P***rof. Dr. George Kowalchuk (External expert), Netherlands Institute of Ecology, Wageningen, the Netherlands. Department of Microbial Ecology.
- **14.45-15.00** ***"Escaping Plato's cave with metagenomics (?)"*** Dr. Tim Urich (AP: MECOMECON), University of Vienna, Austria, Department of genetics in Ecology**.**
- ***15.00-15.15: From molecular inventories to function: aerobic methanotrophs in space and time.*** Prof. Dr. Peter Frenzel (PI; MECOMECON). Max-Planck Institute for terrestrial Microbiology, Marburg, Germany. Department of Biogeochemistry.
- ***15.15-15.30: General discussion.***

[1]: Originally the first presentation was scheduled to be held by Prof. Gerhard Herndl. However, Prof. Herndl had to cancel the day before the session. Instead, a 15 minute presentation was given by Dr. Paul Bodelier on EuroEEFG and on the 4 involved microbial CRP's; DEEP_C, FREDI, MOCA, MECOMECON.

Poster session 31 (Thursday August 23, 15.30-17.30)

200A Influence of (in)organic additions to the function and diversity of prokaryotes in the subtropical North Atlantic Oxygen Minimum Zone Federico Baltar*[1], Thomas Reinthaler[2], Itziar Lekunberri[2], Gerhard J. Herndl[2], Jarone Pinhassi1. *[1]Linnaeus University, Sweden, [2]University of Vienna, Austria*

201A Microbial minorities modulate methane consumption through niche partitioning Paul Bodelier*1, Marion Meima-Franke[1], Kees Hordijk[1], Anne Steenbergh[1], Mariet Hefting[2], Levente Bodrossy[3], Martin Von Bergen[4], Jana Seifert[4] .*[1]Netherlands Institute of Ecology (NIOO-KNAW), Netherlands, [2]University of Utrecht, Netherlands, [3]CSIRO Marine and Atmospheric Research, Australia, [4]UFZ- Helmholtz-Centre for Environmental Research, Germany.*

202A Ubiquity by diversification: Ecological radiation in the species-like freshwater taxon *Polynucleobacter necessarius* (*Betaproteobacteria*) Maria Gadermaier[*1], Jan Jezbera[2], Jitka Jezberova[2], Martin Hahn[1]. *[1]Austrian Academy of Sciences, Institute for Limnology, Austria, [2]Biology Centre AS CR, Institute of Hydrobiology, Czech Republic.*

203A Seasonality and environmental control of freshwater SAR11 (LD12) in a temperate lake (Lake Erken) Friederike Heinrich[*1], Alexander Eiler[2], Stefan Bertilsson[2]. *[1]Uppsala University, Sweden, [2]Uppsala University, Sweden.*

204A Structure and field-scale activity of methane-oxidizing bacterial communities in a landfill-cover soil Ruth Henneberger*, Eleonora Chiri, Martin H. Schroth *ETH Zürich, Switzerland*

205A Trait-based approaches in microbial ecology: a case study on methane oxidizing bacteria testing the phylogenetic signal of functional traits Sascha Krause[*1], Peter van Bodegom[2], Paul L.E. Bodelier[1] .*[1]Netherlands Institute of Ecology, Netherlands, [2]VU University, Netherlands.*

206A Gene expression and diversity of methane oxidizing Proteobacteria in a Sub-Arctic palsa peatland Susanne Liebner[*1], Claudia Lueke[2], Peter Frenzel[2], Mette M Svenning[1] .*[1]University of Tromsø, Norway, [2]Max Planck Institute for Terrestrial Microbiology, Germany.*

207A Biogeography of aerobic methanotrophs in wetland rice fields Claudia Lüke[*1], Adrian Ho[2], Sascha Krause[3], Peter Frenzel[1] .*[1]MPI for terrestrial Microbiology, Germany, [2]Ghent University, Belgium, [3]NIOO-KNAW, Netherlands.*

208A Flow-sorting of uncultivable ultramicrobacteria according to their taxonomic affiliation Stefan Neuenschwander*, Thomas Posch, Jakob Pernthaler, Michaela M Salcher *Limnological Station, University of Zurich, Switzerland.*

209A Assessing protistan grazing on marine prokaryotes in the mesopelagic realm of the Mediterranean Sea Maria Pachiadaki[*1], Andreas Oikonomou[1], William Orsi[2], Virginia Edgcomb[2], Craig Taylor[2], Michail Yakimov[3], Thorsten Stoeck[1] .*[1]University of Kaiserslautern, Germany, [2]Woods Hole Oceanographic Institution, USA, [3]Woods Hole OceanogrCNR-Institute for coastal marine environment, Messinaaphic Institution, Italy*

210A Effect of energy flow on the susceptibility of aerobic methanotrophic communities to disturbance Andreas Reim*, Peter Frenzel *MPI for Terrestrial Microbiology, Germany*.

211A Protein-SIP to elucidate bacterial key players in wetland ecosystems Jana Seifert[*1], Martin von Bergen[1], Paul Bodelier[2] .*[1]UFZ-Helmholtz Center for Environmental Research GmbH, Germany, [2]Netherlands Institute of Ecology (NIOO-KNAW), Netherlands*

212A Bacterial population in the Pelagic zone Abhishek Srivastava[*1], Sarahi L. Garcia[2], Falk Warnecke[2], Hans-Peter Grossart[1] .*[1]Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Department [3]: Limnology of Stratified Lakes, Stechlin, Germany, [2]Jena School for Microbial Communication (JSMC) and Microbial Ecology Group at Friedrich Schiller University Jena, Germany .*

213A A dark and winding road: from microbial sequences to soil biogeochemical fluxes in a global change experiment James T. Weedon[*1], Rien Aerts[1], George A. Kowalchuk[2], Wilfred F.M. Röling[3], Peter M. van Bodegom[1] .*[1]Department of Ecological Science, VU University Amsterdam, Netherlands, [2]Department of Microbial Ecology, Netherlands Institute of Ecology, Wageningen, Netherlands, [3]Department of Molecular Cell Physiology, VU University Amsterdam, Netherlands*

Scientific content

Microbial communities are at the very basis of life on earth, catalyzing biogeochemical reactions driving global nutrient cycles. As yet, they are not on the global biodiversity conservation agenda implying that microbial diversity is not under any threat by anthropogenic disturbance or climate change. However, this maybe a misconception caused by the rudimentary knowledge we have concerning microbial communities in their natural habitats. Despite the genomic era and revolution we are still far away from understanding the functioning of microbial communities *in situ* and especially their individual contributions to biogeochemical reactions. High-throughput sequencing technologies have already yielded a wealth of information on the genetic potential of microbial ecosystems. The availability of thousand of microbial genomes, the possibilities for single cell genomics and proteomics, application of stable isotope incorporation even at single sub cellular level (nanoSIMS) has revolutionized also the functional understanding and perception of the functioning of microbial communities. However, considering the *in situ* functioning, especially on a longer time frame as well as interactions with the environments and other organisms we are largely still looking at a black box situation. The "blackness", however, depends also on the complexity of the ecosystems studied. There are large differences on the level of knowledge between soil/sediment and the aquatic (marine and freshwater) habitats. Yet, important general fundamental issues remain for all habitats:

- **What is the actual functional part of the communities?**
- **What is the value of information not obtained at the relevant scale?**
- **What is the value of assessing microbial communities at the kingdom level?**
- **Do we need to know community composition (genotype) and species characteristics (phenotype) to understand ecosystem functioning?**

Within EuroEEFG there are 4 CRP's that address the issues raised above using functional genomics tools. The CRPS's cover marine (MOCA, DEEP_C), freshwater (FREDI) and terrestrial habitats (MECOMECON). In the contributed session CRP's members presented EuroEEFG research thereby also giving their view on the application of functional genomics tools and the knowledge it has

generated on environmental microbial communities in marine freshwater and terrestrial habitats. Metagenomic and scale issues were highlighted.

Abstracts

Exploring trophic interactions in the microbial web of the deep-sea Thorsten Stoeck[*1], Maria Padiadaki[1], Viriginia Edgcomb[2], Michail Yakimov[3]. [1]*University of Kaiserslautern, School of Biology, Germany,* [2]*Department of Geology and Geophysics, Woods Hole Oceanographic Institution, USA,* [3]*Institute for Coastal Marine Environment, IAMC-CNR, Italy*

The deep ocean covers two-thirds of our planet and teems with microbial life. Understanding the roles of deep-sea microbial communities is therefore essential for understanding global biogeochemical cycling, which in turn is pivotal to all other forms of life. The breakthrough discovery that marine bathypelagic realms are significant zones of autotrophic CO2 fixation, i.e. areas of dark ocean primary production, is perhaps the most exciting application of modern molecular approaches in the field of deep-sea microbiology. Considering that deep-sea environment represents the biggest ecosystem on our planet, it is surprising that the contribution of bathypelagic chemolithoautotrophic production to the microbial food web and to global carbon cycling in general has not been studied yet. After all, it represents a major CO2 sink on Planet Earth. Recently, members of Marine Group I of Crenarchaeota have been identified to be involved in deep-sea chemolithotrophic production of organic carbon. Estimates of the global archaeal dissolved inorganic carbon (DIC)-fixation rate are 4-8 x 1011 kg C yr-1. This carbon represents a substrate for a largely unknown deep-sea food web including prokaryotes, protists, and metazoa. Marine Crenarchaeota, being very abundant in the deep-sea, seem to play a pivotal role in the ocean's nitrogen cycle: they are capable to grow using ammonia as energy and CO2 as carbon source. The DEEP_C consortium in the framework of the EUROCORES program Ecological and Evolutionary Functional Genomics (EuroEEFG) aims to directly link deep ocean dark CO2 fixation and the identity of the organisms or assemblages involved in this process. As part of this consortium the presented individual project aims to elucidate the role of phagotrophic protists in shaping archaeal deep-sea communities and affecting archaeal-intermediated dark carbon fixation. In a first step, we explore new technologies to adequately study protistan phagotrophy in deep-sea habitats, which I will present and discuss in my talk.

From communities to single cells: finding functional coherence in the lake microbiome Stefan Bertilsson *Uppsala University, Sweden*

Freshwater bacterial communities are highly diverse and dynamic in time and space. With limited cultivation success, studies that aim for a better understanding of the ecology, diversity and functioning of such complex communities are typically pursued with molecular methods, taking advantage of biomarker genes that can be used as proxies for either broadly or narrowly defined taxonomic groups. Synoptic studies of such biomarkers from lakes have thus far demonstrated that freshwater bacteria are phylogenetically distinct from bacteria found in other biomes. Furthermore, the freshwater lake microbiome appears to be dominated by a limited number of closely related and globally dispersed bacterial groups (tribes) that respond in contrasting ways to environmental driver variables. However, it still not clear to what extent such uncultured monophyletic "tribes" represent populations with shared ecology and function in the lake ecosystem. In the present talk I will review alternative approaches to experimentally explore this framework in order to find and decipher ecological and functional coherence within complex aquatic bacterioplankton communities. Examples will be from some of the most abundant lineages in this biome: e.g. alf-V featuring the freshwater sibling group of marine SAR11 and the ubiquitous freshwater Actinobacteria acI. First, highly resolved population-tracking in time and space enabled by parallelized next generation sequencing of biomarker genes can reveal co-occurrence patterns at variable levels of phylogenetic resolution with shared or contrasting ecology inferred. Second, substrate-tracking single cell approaches combined with taxonomic identification by fluorescence in situ hybridization can resolve metabolic differences in pre-defined closely and distantly related populations. Finally, taxonomy-guided genome sequencing of single cells and targeted metagenomic analysis can uncover the genomic background to ecological and functional differentiation. The advantages and limitations of these alternative approaches will be discussed and compared while addressing the broader question of whether or not function can be inferred from taxonomy.

What can we learn on ecology of the uncultivated acI lineage of freshwater *Actinobacteria* from genomic analyses? Hans-Peter Grossart[*1], Sarahi L. Garcia[2], Katherine D. MacMahon[3], Manuel Martinez-Garcia[3], Abhishek Srivastava[3], Alexander Sczyrba[3], Ramunas Stepanauskas[4], Tanja Woyke[5], Falk Warnecke[3] .[1]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Germany,* [2]*FSU Jena/ Institute for Microbiology, Germany,* [3]*, USA,* [4]*Bigelow Laboratory for Ocean Sciences, USA, 5US DOE Joint Genome Institute, USA*

Actinobacteria within the acI lineage are often numerically dominating freshwater ecosystems where they can contribute >50% of the bacteria in the surface water. However, they are uncultured to date. Wethus set out to use single cell genomics to gain insights into their genetic make-up to learn about theirphysiology and ecological niche. Single cells were isolated by fluorescence-activated cell sorting, wholegenome amplified using Phi29 DNA polymerase, and the resulting single amplified genomes screened by16S rDNA sequencing. Two highly abundant acI clade representatives were selected for shotgun genomicsequencing followed by single cell genome assembly, annotation and data analysis. Here, we report on genome comparison of 2 closely related *Actinobacteria* of the acI clade in respect to their metabolic functions in relation to contrasting environmental conditions. We will present hints on their metabolic potential regarding primary metabolism,

photometabolism, stress responses, transporters, and other metabolic features. This project reveals the possibilities and limitations of single cell genomics for microbial species that defy cultivation to date.

Living together apart: soil metagenomics and microbially relevant scales.

Kowalchuk George, *Netherlands Institute of Ecology, Netherlands*

Soil habitats harbor an incredible amount of biological diversity, and our appreciation of this diversity has increased tremendously with the influx of high-throughput cultivation-independent and metagenomics approaches. However, this great species richness raises both practical and theoretical problems. On the practical side, the vast diversity of soil habitats hampers our ability to disentangle relevant microbial players, activities and interactions. From the conceptual perspective, we still lack a general understanding of the forces that drive and maintain soil's vast microbial diversity. Similarly, we still have a poor grasp of what constitutes the functional unit in soil microbial communities or the rules of community assembly. With these limitations in mind, it is proposed that soil-borne microbial communities can best be interrogated in manageable units of diversity, to allow for the study of meaningful microbial interactions. These manageable units of diversity can either be achieved by zooming in a particular subset of the community via labeling and enrichment methods or by focusing in on very small samples sizes or discrete soil compartments. Coupling such directed or micro-scale approaches with the power of high-throughput sequencing methodologies provides the necessary depth of information required to extract more meaningful units of interactions. These perspectives are demonstrated via a series of studies that seek to examine the soil metagenome via meaningful and manageable components of the total soil microbial community.

Escaping Plato´s cave with meta-'omics' ?

Tim Urich *University of Vienna / Department of Genetics in Ecology, Austria*

The identity, functioning and interactions of microbes *in the wild* are often difficult to characterise, due to the small size of the microbes and the huge diversity in environmental communities. Microbial ecologists can therefore be seen like the prisoners in Plato´s cave allegory, trying to understand the world by observing shadows on the walls of a cave.

The combination of the metagenomics toolkit with new sequencing technologies has promised a deeper understanding of environmental microbial communities (and thus may lead ways out of the cave). I will present two recent examples of the application of metatranscriptomics from own studies, that were used to (1) link the identity and function of a yet uncultured euryarchaeal clade in bovine rumen, and to (2) characterise complex properties of communities, like trophic interactions in the soil microbial foodweb. Opportunities, pitfalls and novel perspectives of the meta-'omics' approach will be discussed.

From molecular inventories to function: aerobic methanotrophs in space and time

Peter Frenzel *Max-Planck-Institute for Terrestrial Microbiology, Germany*

Aerobic methane-oxidizing bacteria (MOB) provide a key service in global ecology reducing significantly the amount of the greenhouse gas methane emitted from sources like wetlands and rice fields, and forming the only biological sink for atmospheric methane in upland soils. MOBS are specialist using a very restricted substrate range. They are accessible targeting a gene encoding a subunit of the key enzyme, the methane monooxygenase. Based on 5000 sequences covering the known *pmo*A diversity we defined 40 major *pmoA*-like lineages. Many of these lineages correlate with ecological patterns, e.g. several lineages contain exclusively sequences recovered from marine or saline environments. Furthermore, specific sequences were nearly exclusively retrieved from soils consuming methane at atmospheric concentration. Here we focus on the ecology of MOB in rice fields, one of the major methane sources and one of best studied methanotroph environments, too. Our main questions are (i) what allows the coexistence of up to 30 species-equivalent operational taxonomic units in a single paddy, and (ii) does this diversity translate into resistance and/or resilience? Our molecular analysis focused on *pmoA*. Methods include tRFLP, a diagnostic microarray, qPCR, sequencing and pyrosequencing. We developed an error-tolerant classifier to identify and analyze next generation sequences. Studies were performed on samples taken from rice paddies situated between 8° E and 121° E, and 8°S and 45°N. This dataset includes a chronosequence covering rice paddies cultivated from 50 up to 2000 years. For the experiments, we use microcosms constructed with paddy soils form Italy and China, respectively. Throughout the regions studied, methanotroph communities were qualitatively nearly identical. However, the relative community composition was significantly different showing a pronounced distance-decay relationship. Environmental factors like soil pH and agricultural practice explained only a minor fraction of measured dissimilarity. Length of rice cultivation, however, had an effect on population dynamics and activity of a particular subgroup, if samples were incubated under favorable conditions. Hence, continuous rice agriculture may not only shape the community, but also modify the micro-environment in a way enabling faster growth and higher activity of a subpopulation. Protistan grazing is shaping the community, too, but should be a natural factor occurring everywhere. The activity of MOB living at the surface of flooded soils is diffusion-limited, and in the very narrow zone where they can be active, concentrations of the substrates methane and oxygen are nearly the same regardless how much methane is produced. The source strength, however, i.e. the rate of methanogenesis, did influence the community composition of methanotrophs. Controls of methanotrophs and methane oxidation at the oxic root-soil interface are less clear, but the genotype of rice cultivars does influence methanotroph community structure, but again quantitatively and not qualitatively. In summary, we see in the microcosms experiments a separation of MOB at the spatial microscale and in time, but most important, between active state and the seedbank. With methanotrophs surviving for decades in dried soils or anoxic sediments, the rate of extinction is low. The seedbank is also a prerequisite for maintain functioning in a fluctuating environment. Severe losses, heat stress, and even pasteurization could be compensated for without striking effects on community structure. Care should be taken, however, applying the lessons from paddy soils to low-methane environments: energetically limits may prohibit loss compensation, if methane and energy flow through a methanotroph community becomes too low.

General discussion

The final 15 minutes of the session was dedicated to a general discussion between the speakers and the audience. The discussion evolved mainly regarding the originally proposed discussion points of "Studying the relevant phylogenetic level" as well as the "relevant scale". The often taken approach in metagenomic studies, investigating microbial communities top-down, at higher phylogenetic levels was judged not to be very useful to understand the functioning and controlling factors of microbial communities. Especially, the examples presented from fresh and marine aquatic systems demonstrated that there are large ecological differences below the species level. The total metagenomic approach including non relevant microbial scales, is very helpful in assessing the genetic potential and the cataloging of the functional and phylogenetic diversity of microbial communities. It will also generate hypotheses of possible interaction and mechanistic relationships. However, there was consensus that work on single cell level and work focusing on microbial communities on relevant scales, in combination with ecophysiological studies on relevant organisms in the laboratory is crucial to understand what is going on in the "black box". Single cell genomics, proteomics, stable isotope labeling, isolation studies are all necessary more than ever and the effort spent at the moment in metagenomic and large scale sequencing studies should be diverted more to work with actual intact microbes. Consensus was reached that much more manipulative experimental studies in laboratory as well as under field conditions should be performed where biogeochemical and genomic aspects at various levels of organization are combined to come to a holistic understanding of microbial communities. Hence, platforms like EuroEEFG are ideal for these purposes and served this cause by training young researchers who will carry on this research approach and attitude.

Poster session

The oral contributed session was directly followed by the EuroEEFG posters session. 13 posters were presented by PhD students and post-docs on the work they have been carrying out within the EuroEEFG framework. The abstracts of the posters are attached below in the annex of this report.

Results and impact on the EUROCORES programme

The ISME symposium was visited by 2200 participants among which the most influential scientist in the field of microbial ecology as well as many young investigators as students from more than 60 countries. The oral contributed session was attended by approximately 150 attendants, while the 13 posters where available for viewing for 2 full conference days. Hence, the exposure and dissemination of the ESF-coordinated research was tremendous. The first 15 minutes of the oral session were dedicated to presenting the structure and the rationale of the EUROCORES programme in general and EuroEEFG more specifically. Hence, the message of the multidisciplinary, high level cooperations and scientific networks where EUCORES is all about was conveyed to a very large and wide audience. The block presentation as oral session followed directly by a posters session which was also physically arranged together represented the EuroEEFG as it should, namely as cooperating scientist dedicated to create synergy for the advancement of science.

Besides the presentation of the science, 12 PhD students and post-docs within EuroEEFG were giving the opportunity to present and discuss their work, to interact with the most important scientist in their

field and to enlarge their scientific network, which is of vital importance for their career. Hence, beside the dissemination of ESF and the science supported the event was also had a great impact on the training and career development of the young scientist within EuroEEFG.

Annex 1: Abstracts of poster session

200A Influence of (in)organic additions to the function and diversity of prokaryotes in the subtropical North Atlantic Oxygen Minimum Zone Federico Baltar[*1], Thomas Reinthaler[2], Itziar Lekunberri[2], Gerhard J. Herndl[2], Jarone Pinhassi[1] .*[1]Linnaeus University, Sweden, 2University of Vienna, Austria*

Ocean surface temperatures are predicted to rise, increasing stratification and enhancing the natural existing Oxygen Minimum Zones, which could favor chemoautotrophic processes. Although Oxygen Minimum Zones account for only 0.1% of the global ocean volume, 30–50% of the oceanic N loss is estimated to occur therein, playing a crucial role in controlling the oceanic nutrient inventory. Mesophilic marine Crenarchaeota Group I have been found to be autotrophic nitrifiers. A potential chemolithoautotrophy was recently suggested for uncultured Proteobacteria lineages in the dark ocean, related to use of alternative energy sources like inorganic N and S compounds. However, the potential influence of those compounds on the prokaryotic community has not been empirically confirmed by metabolic rates measurement in the dark open ocean. We studied the changes in prokaryotic heterotrophic/autotrophic functioning and diversity in response to organic carbon (pyruvate + acetate) and inorganic N (ammonium) and S (thiosulfate) compounds with water from the subtropical North Atlantic Oxygen Minimum Zone. To do this, we tracked the changes in heterotrophic production, dark dissolved inorganic carbon fixation together with prokaryotic abundance, extracellular enzymatic activities, and prokaryotic community composition (454 tag pyrosequencing of 16S rDNA) in three microcosms experiments. Oxygen Minimum Zone's prokaryotic community was metabolically stimulated in all experiments by ammonium (i.e., >2 fold increase in dissolved inorganic carbon fixation) and by organic carbon compounds (that is prokaryotic abundance and all metabolic rates were enhanced >10-fold, including dissolved inorganic carbon fixation), but not by thiosulfate. These results suggest an important role of dark dissolved inorganic carbon fixation in the carbon flux of the subtropical Atlantic related to ammonium availability and/or organic matter pulses. Alkaline phosphatase was the metabolic rate more stimulated when organic carbon compounds were added, indicating a key relation between this enzymatic activity and the organic carbon pool in the dark ocean. Only the addition of organic carbon compounds (but not ammonium or thiosulfate) changed the Oxygen Minimum Zone prokaryotic community structure, where Archaea cells decreased and some Alteromonodales, Rhodobacteraceae, Oceanospirillales and Vibrionaceae increased. All the taxa that increased in relative abundance originally belonged to the rare biosphere, selectively emerging depending on the original assemblage settings, and notably contributing to the community's biogeochemical and ecological roles.

201A Microbial minorities modulate methane consumption through niche partitioning Paul Bodelier[*1], Marion Meima-Franke[1], Kees Hordijk[1], Anne Steenbergh[1], Mariet Hefting[2], Levente Bodrossy[3], Martin Von Bergen[4], Jana Seifert[4] .*[1]Netherlands Institute of Ecology (NIOO-KNAW), Netherlands, [2]University of Utrecht, Netherlands, [3]CSIRO Marine and Atmospheric Research, Australia, [4]UFZ- Helmholtz-Centre for Environmental Research, Germany*

The anomalies in atmospheric methane concentrations in late twentieth century, including the renewed increase since 2007 have been proposed to be caused by changes in microbial methane cycling in wetland ecosystems. Aerobic methane oxidizing bacteria (MOB) play a vital role in the mitigation of wetland methane emissions by degradation of the greenhouse gas CH4. However, the diversity of MOB communities and traits of the microbes involved are not taken into consideration when assessing potential sources of variation in the global methane budget. The aim of this study is to assess the role of MOB diversity and ecology in methane consumption. To this end methane consumption and MOB communities were investigated in a hydrological gradient situated in a river floodplain along the river Rhine, the Netherlands. Methane consumption was assessed *in vitro* as well as in intact cores and MOB communities were assessed using *pmoA*-based micro array and QPCR on mRNA as well as DNA. The *in situ* active MOB were indentified using Stable Isotope Probing (SIP) of lipids and proteins. The flooding regime in the Rhine floodplain established a distinct CH4 consumption pattern which was reflected in the distribution of subgroups of γ-proteobacterial MOB. Diversity index as assessed by micro array and methane consumption was positively correlated which was caused by the relative abundance of γ-proteobacterial MOB relative to α-proteobacterial MOB, suggesting niche partitioning between these subgroups. SIP of proteins and lipids as well as mRNA analyses showed that γ-proteobacterial MOB (type Ia and b) were responsible for the activity. The relative abundance of specific subgroups which can be regarded as being part of the "rare" biosphere present in these soils controlled CH4 consuming activity which makes it evident that knowledge on the microbial community composition is necessary to predict effects of environmental change on methane cycling in wetland habitats. Next to this, our results show that the interpretation of microbial Biodiversity-Ecosystem functioning experiments can be biased by lack of environmental heterogeneity and associated niches and by inability to assign species to function.

202A Ubiquity by diversification: Ecological radiation in the species-like freshwater taxon *Polynucleobacter necessarius* (*Betaproteobacteria*) Maria Gadermaier[*1], Jan Jezbera[2], Jitka Jezberova[2], Martin Hahn[1]. *[1]Austrian Academy of Sciences, Institute for Limnology, Austria, [2]Biology Centre AS CR, Institute of Hydrobiology, Czech Republic*

The taxon *Polynucleobacter necessarius* represents abundant planktonic bacteria contributing up to 70% (on average 20%) to bacterial numbers of freshwater bacterioplankton. This group of bacteria is characterized by sharing almost identical 16S rRNA genes (≥ 99% sequence similarity), as well as by a cosmopolitan and ubiquitous distribution in freshwater systems.

They were detected in a broad spectrum of freshwater habitat types ranging from Arctic and Antarctic to tropical lakes and ponds, as well as over a pH range of at least 3.9 to 8.5. Besides free-living planktonic strains, this taxon also includes obligate endosymbionts of ciliates affiliated with the genus *Euplotes*. We tested if the broad habitat range and the ubiquitous distribution of *P. necessarius* bacteria resulted from a generalist adaptation of strains or from ecological diversification into distinctly adapted and specialized lineages (ubiquity by diversification hypothesis). We tested by cultivation-independent (e.g. reverse line blot hybridization) and cultivation methods if *P. necessarius* subgroups differ in (i) their distribution across environmental gradients (e.g., pH of freshwater systems), (ii) biogeographic distribution, and (iii) the presence/absence of putative adaptive genes. It was observed that particular environmental (e.g. pH) or climatic conditions excluded the presence of certain *P. necessarius* subgroups. For instance, some subgroups were found to be restricted to acidic habitats, while others were exclusively detected in neutral or alkaline systems. Furthermore, a subgroup was identified, which is restricted to habitats located in the tropical climatic zone. Screening of 203 *P. necessarius* strains for the presence of a key gene (pufM) of anoxygenic photosynthesis resulted in detection and sequencing of the gene in one-third of the strains. These results clearly support the ubiquity by diversification hypothesis. The taxon *P. necessarius* seems to represent a complex group of ecotypes significantly differing in ecological adaptation. Thus, this group of bacteria resembles ecologically a cryptic species complex, which cannot be resolved by analyzing 16S rRNA gene sequences.

203A Seasonality and environmental control of freshwater SAR11 (LD12) in a temperate lake (Lake Erken) Friederike Heinrich[*1], Alexander Eiler[2], Stefan Bertilsson[2]. [1]*Uppsala University, Sweden,* [2]*Uppsala Univeristy, Sweden*
The SAR11 clade is ubiquitous in aquatic environments and often accounts for a large portion of the total bacteria in planktonic environments. In freshwater lakes, the clade is represented by tribe LD12, a group which is phylogenetically distinct from the marine SAR11. In this study we explored the ecology of LD12 in a temperate dimictic lake, Lake Erken, by analyzing its seasonal dynamics with group-specific quantitative PCR and 454 pyrosequencing of the 16S rRNA gene. We demonstrate that LD12 can be as numerous in freshwater bacterioplankton as their more widely known marine SAR11 siblings. They also feature strong seasonality in their contribution to the total bacterioplankton. Over the course of a year, LD12 made up 2.8-40% of the total bacterial 16S rRNA pool (mean 17%) with pronounced peaks in both summer and late fall. Except in spring, LD12 was by far the dominant Alphaproteobacteria, contributing on average 72% of the 16S rRNA amplicons within this class. The LD12 population was dominated by a single ribotype that persisted over the entire annual cycle, suggesting low local divergence, at least at the phylogenetic resolution that can be accessed with rRNA genes. Partial least square projections to latent structures revealed positive correlations between the relative abundance of LD12, available nutrients (phosphate, ammonia, nitrate, and silica), and water transparency. The relative abundance of LD12 was lower during periods characterized by high phytoplankton biomass and low availability of free inorganic nutrients. Based on these observations we propose that LD12 represents an abundant group of freshwater bacteria that are poor competitors during periods of high phytoplankton productivity coupled with an associated release of labile organic compounds, but rather thrive under conditions of high availability of inorganic nutrients. Nevertheless, similar to the marine SAR11 sibling group, local LD12 populations in different lakes appear to respond in contrasting ways to nutrient availability, pointing to either ecological divergence within the tribe or variations in the interplay between environmental driver variables.

204A Structure and field-scale activity of methane-oxidizing bacterial communities in a landfill-cover soil Ruth Henneberger*, Eleonora Chiri, Martin H. Schroth *ETH Zürich, Switzerland*
Methane (CH4) is a potent greenhouse gas contributing strongly to global climate change. Aerobic methane-oxidizing bacteria (MOB) play an important role in mitigating CH4 emission to the atmosphere by utilizing CH4 as sole energy and carbon source. MOB are ubiquitous in soils and particularly abundant in CH4-rich habitats, such as landfill-cover soils. Detection and identification of MOB is generally based on the pmoA gene, encoding a subunit of the enzyme methane monooxygenase. However, cellular membranes of MOB also contain characteristic phospholipid fatty acids (PLFA) that can be used for MOB differentiation. Laboratory-based stable-isotope probing (SIP) of PLFA has been widely used to identify active MOB communities. These results are generally difficult to extrapolate to the field, highlighting the importance of complementary field-based approaches. In this study, we investigated MOB in a Swiss landfill-cover soil by combining activity measurements in the field (gas push-pull tests, GPPT) with molecular analyses (cloning, terminal restriction-length polymorphism (T-RFLP) and quantitative PCR) of the pmoA gene. In addition, a novel field-based SIP approach was developed which combines PLFA-SIP with GPPTs. During this SIP-GPPT, 13CH4 is used in the injected gas mixture to label active MOB at the field-scale while simultaneously quantifying turnover rates. The MOB community present at the study site was highly active, with potential CH4 oxidation rates ranging from 1.8 to 58.2 mmol CH4 (L soil air)-1 d-1. Significant lateral variation in activity was observed, and oxidation rates were positively correlated with mean CH4 soil gas concentrations ($P<0.01$), MOB abundance ($P<0.05$), and MOB diversity (weak correlation, $P<0.17$). MOB communities at different locations were highly diverse, yet Type Ia MOB were dominant, and novel pmoA sequences were discovered. Type II MOB were mainly detected in deeper soil layers with lower nutrient and higher CH4 concentrations. Substantial differences in MOB community structure were observed between one high- and one low-activity location, and Type II MOB-specific pmoA sequences were only detected at the high-activity location. The SIP-GPPT method was applied to selected locations with diverging activities, to identify active members of the MOB community. Incorporation of 13C into characteristic MOB fatty acids (FAs) was clearly demonstrated by increased δ13C values (up to ~1500 ‰) compared to natural background values. The amount of 13C incorporation into biomass was positively correlated with CH4 oxidation rates. In general, FAs C14:0, C16:1ω8, C16:1ω7 and C16:1ω6 (type I MOB) showed highest label incorporation, while FAs C18:1ω8 and C18:1ω7 (type II MOB) were only labelled at the high-activity locations. The combination of DNA-based analyses with field-based activity measurements and the SIP-GPPT applied in this study provided unique insights into the MOB community present in this landfill-cover soil. Our findings show a clear dominance of Type I MOB, and suggest that Methylosarcina and closely related MOB are key players in CH4, while the results also indicate that MOB abundance and community structure are driving factors in CH4 oxidation at this landfill.

205A Trait-based approaches in microbial ecology: a case study on methane oxidizing bacteria testing the phylogenetic signal of functional traits Sascha Krause[*1], Peter van Bodegom[2], Paul L.E. Bodelier[1]. [1]*Netherlands Institute of Ecology, Netherlands,* [2]*VU University, Netherlands*

Microbes form a major part of earth biomass and biodiversity and have important impacts on biogeochemistry and ecosystem processes. Since microbial communities and their functioning can be sensitive to anthropogenic and non-anthropogenic disturbances, efforts have been made to understand the relation between microbial diversity and ecosystem functioning. An ultimate goal is to predict how microbial communities and their functioning respond to environmental changes. In 2008, Allison and colleagues suggested a promising conceptual framework to incorporate microbes into process models through their abundances and physiological responses to disturbance. Nonetheless, the framework is so far only hypothetical and empirical data are needed to prove whether there is a link between microbial phylogeny, physiological traits, and disturbance responses. Here we focus on aerobic methane oxidizing bacteria (MOB) as a model system because they have a well-characterized physiology, can be targeted specifically with molecular tools and catalyze an important ecosystem function. We investigated the relationship between MOB phylogeny and methane oxidation kinetic parameters as functional traits. If a link between phylogeny and functional traits is present, this would increase the predictability of microbial processes substantially. We calculated the phylogenetic signal, that is the statistical dependence among species traits through their phylogenetic relationship. We applied methods well established in evolutionary biology. For discrete data we used Mesquite and for continuous data the software package Picante as implemented in the statistical software R. First, a literature study was performed to generate a representative data set of morphological, biochemical and physiological traits assumed to be related to phylogeny. This data set was used to test phylogenetic signal methods for microbial ecology approaches. Second, the only known comprehensive data set on methane oxidation kinetics from Knief and colleagues (2005) has applied to identify the degree of phylogenetic signal in methane oxidation parameters. All analyses were performed both on the 16s rRNA gene and the *pmoA* gene level. The *pmoA* gene encodes a subunit of methane monooxygenases which are involved in the first step of methane oxidation and has been intensively used for the detection of MOB. Initial results demonstrated indeed a phylogenetic signal of morphological (internal membrane structure), physiological (optimal growth temperature, carbon assimilation pathway) and biochemical (phospholipid fatty acids, GC content) traits. For the functional traits of MOB, there was a phylogenetic signal detected for the methane oxidation kinetics (that is Km) and methane required for maintenance, albeit much weaker than for non-kinetic traits. In conclusion, these results indicated that kinetics of methane consumption can be used as trait that is connected to phylogeny and can thus be considered in trait-based biodiversity-ecosystem functioning models. However, more experimental data is necessary to verify these initial results.

206A Gene expression and diversity of methane oxidizing Proteobacteria in a Sub-Arctic palsa peatland Susanne Liebner[*1], Claudia Lueke[2], Peter Frenzel[2], Mette M Svenning[1]. [1]*University of Tromsø, Norway,* [2]*Max Planck Institute for Terrestrial Microbiology, Germany*

Methane oxidizing (methanotrophic) Proteobacteria (MOP) mitigate the emission of methane globally. Methane is a relevant greenhouse gas, so that the oxidation of methane is an important ecosystem function. In palsa peatlands, methane emissions are high from flarks, lawns, and ponds but negligible from the elevated palsas which contain of a permanently frozen core. Palsas typically occur in the Sub-Arctic around the 0°C isotherm (annual mean). They are very sensitive towards a changing climate and constitute an appropriate system to monitor how methane fluxes change along with permafrost degradation. We studied gene expression and community structure of MOP in different sites of palsa succession in an acidic (pH 3.5-4.2) Sphagnum dominated peatland from northern Norway. The pmoA gene which encodes the α-subunit of the particulate methane monooxygenase and the mmoX gene, specific for the soluble methane monooxygenase and encoding the α-subunit of its hydroxylase, were used as methanotroph specific functional marker genes. We performed pyrosequencing with tagged pmoA specific primers as well as pmoA, mmoX, and nifH clone library analysis on DNA and cDNA. Transcribed pmoA mainly clustered with peat originating Methylocystis sequences, and with Methylocapsa and Methylomonas. Transcribed mmoX was found in-situ and was affiliated with Beijerinckiaceae and Methylomonas. Transcription of mmoX indicates that the soluble methane monooxygenase is of ecological relevance in this low pH peatland but its function in the environment needs to be elucidated. Methanotrophs were also among those prokaryotes transcribing nifH indicating nitrogen fixation of methanotrophs in this highly oligotrophic peatland.

207A Biogeography of aerobic methanotrophs in wetland rice fields Claudia Lüke[*1], Adrian Ho[2], Sascha Krause[3], Peter Frenzel[1] .[1]*MPI for terrestrial Microbiology, Germany,* [2]*Ghent University, Belgium,* [3]*NIOO-KNAW, Netherlands*

Understanding the mechanisms underlying biodiversity is important to predict ecosystem responses to environmental changes. For macroorganisms, biogeographical patterns have been studied extensively; however, research on microbial dispersal is still in its infancies. In our work, we focused on the spatial distribution of aerobic methane oxidizing bacteria (methanotrophs) in wetland rice fields. Methanotrophs can be linked to a defined ecosystem function and their diversity has been studied extensively. Furthermore, due to the low complexity (one major plant species, similar agricultural management), paddy fields represent an ideal model habitat as environmental differences are reduced to a minimum. We used the pmoA gene (encoding a subunit of the methane monooxygenase) as marker for methanotroph diversity in paddy fields located in Italy, China and distributed across the Indonesian islands Java and Sumatra. *PmoA* diversity was analyzed using T-RFLP, microarray and pyrosequencing. We defined 40 major phylogenetic pmoA-like lineages, based on a set of over 5000 sequences covering the to-date known *pmoA* diversity. Interestingly, many of these lineages describe environmental distribution pattern for example several lineages consist entirely of sequences recovered from marine or saline environments (Methylomicrobium japanense-like, deep sea-1 to -5). Furthermore, sequences clustering within the upland soil clusters (USC-alpha, USC-gamma, JR-2, and JR-3) were nearly exclusively retrieved from soils consuming methane at atmospheric concentration. In addition, the main lineages within type Ib methanotrophs (freshwater sediment-1 and -2) are highly dominated by rice paddy and freshwater lake sequences. Out of the 40 lineages, Methylocystis-like (type II methanotrophs)

and freshwater sediment lineage-2 (type Ib) formed two core lineages distributed in high abundance through all paddy soils investigated here. Remarkably, despite deep sequencing, no upland soil clusters could be detected in any of the paddy soils. Though, these sequences were found to be highly abundant in an adjacent forest soil. Distance-decay patterns were analyzed at the local (within one paddy field), regional (across Italian paddy fields with 20 km distance) and global scale (across paddy fields located within 12,000 km distance). Whereas no spatial distribution of methanotrophs could be observed at the field scale, significant correlations were found at the regional and global scale. These patterns could not be explained by the measured environmental parameters. However, differences in community composition were largely attributed to different abundances, but not to different lineages. PmoA core lineages were analyzed in detail for potential phylogenetic clustering according to geographical location within a lineage. However no such grouping could be detected. Thus, despite a growing number of studies assumes dispersal limitation for bacteria, we cannot confirm this observation for wetland rice methanotrophs. We hypothesize that distance-decay patterns were created by historical contingencies rather than dispersal limitation followed by speciation events.

208A Flow-sorting of uncultivable ultramicrobacteria according to their taxonomic affiliation Stefan Neuenschwander*, Thomas Posch, Jakob Pernthaler, Michaela M Salcher *Limnological Station, University of Zurich, Switzerland*

Ultramicrobacteria numerically dominate the bacterioplankton in many natural aquatic systems. Understanding their genetic properties is therefore of high interest, however, cultivation of those microbes is still very challenging. Separating phylogenetically defined populations from their natural communities prior to (meta-) genomic analysis could therefore be a promising alternative. Insights into the physiological capabilities of defined bacterial populations are highly needed to understand their role in natural habitats. One way to achieve such a physical separation is to combine fluorescence in situ hybridisation and flow cytometry. Such protocols are available since the nineties and have been successfully applied to samples originating from nutrient rich environments. Limited signal intensities however hindered a broad application to samples from natural aquatic environments. The introduction of CARD-FISH based protocols partly solved this problem, but when applied to ultramicrobacteria, insufficient signal to noise ratios are still an issue. We aimed to improve the sensitivity of such a protocol by complementing it with antibody mediated secondary tyramide signal amplification. For evaluation we used samples from the oligo-mesotrophic Lake Zurich and a oligonucleotide probe specific for the highly abundant but so far uncultivable LD12 (SAR11 IV) cluster of *α-Proteobacteria*. Compared to previously existing protocols we observed superior signal to noise ratios. High purity flow sorting of LD12 ultramicrobacteria is now possible with this improved protocol making downstream applications such as (meta-) genomic analyses feasible.

209A Assessing protistan grazing on marine prokaryotes in the mesopelagic realm of the Mediterranean Sea Maria Pachiadaki[*1], Andreas Oikonomou[1], William Orsi[2], Virginia Edgcomb[2], Craig Taylor[2], Michail Yakimov[3], Thorsten Stoeck[1] .*[1]University of Kaiserslautern, Germany, [2]Woods Hole Oceanographic Institution, USA, [3]Woods Hole OceanogrCNR-Institute for coastal marine environment, Messinaaphic Institution, Italy*

Grazing by phagotrophic protists, and viral-mediated lysis are currently considered the main sources of microbial mortality in aquatic ecosystems. As a consequence, protist predators may considerably affect major prokaryote-mediated biogeochemical cycles and processes. Despite its ecological importance, information on these interactions is lacking. To estimate the impact of phagotrophy on deep-sea bacterioplankton, a fluorescently labelled prokaryotes (FLPs) tracer technique was used. During the R/V Atlantis cruise in late 2011, short-term grazing experiments were conducted in four different depths (40m, 200m, 500m and 950m) with the use of DEEP-SID sampler, built at the Woods Hole Oceanographic Institution. This device conducts incubation studies where samples are manipulated at depth under in situ conditions and was used to mix water samples with FLPs in situ. The FLPs-seawater incubation was subsampled every 10 minutes (from time zero to 60 minutes), and mixed with fixative. Total prokaryotic and eukaryotic abundances and the number of ingested FLPs per eukaryote were counted by epifluorescence microscopy. The initial results show a high prokaryotic turnover rate by the heterotrophic nanoflagellate community in the euphotic zone, removing daily more than 50% of standing prokaryote stock. The abundances of prokaryotes, microscopic eukaryotes and the protistan grazing declined substantially with depth within the mesopelagic water column. To our knowledge this is the first time that an in situ grazing experiment is conducted in the mesopelagic realm.

210A Effect of energy flow on the susceptibility of aerobic methanotrophic communities to disturbance Andreas Reim*, Peter Frenzel *MPI for Terrestrial Microbiology, Germany*

At the surface of water logged soils and sediments, methanotrophic bacteria thrive on the oxidation of methane. Methane is supplied from the anoxic bulk soil below, and oxygen from the overlying water body. The spatial separation of the two substrate sources leads to the formation of overlapping gradients of methane and oxygen, with minima where the methanotrophs are active. Irrespective of methane source strength, the substrate concentrations at the interface are comparably low. Only the location of the oxic-anoxic interface and, hence, the flux at the interface is affected. The energy flow as a potential factor affecting the methanotroph community was neglected so far. Here we demonstrate that the energy flow is influencing methanotrophic community's structure as well as population dynamics. By diluting a native into a γ-ray sterilized soil (1:40) we simulated a severe die-off of the microbial community, including methanotrophs. This disturbance allowed us to follow the re-establishment of a methanotroph community as a function of energy flow. Community structure was analyzed by t-RFLP, a diagnostic microarray, and by competitive RT-PCR targeting the *pmoA* gene, a functional and phylogenetic marker for methanotrophs. *pmoA* transcripts served as a proxy for species-specific activity. In general, *Methylobacter* related methanotrophs (type I) recovered faster under high energy flow. *Methylocystis* and *Methylosinus* methanotrophs (type II) were not significantly affected by the energy flow, but rather by disturbance in general. Hence, higher energy flows seem to select for a more resilient type I dominated community. However, in the undisturbed control incubations, we observed a shift from type I to type II methanotrophs under high energy flows. We hypothesize this to be the

result of a higher resistance of type II methanotrophs to grazing. This is consistent with earlier experiments on the susceptibility of methanotrophs to grazing, where type I methanotrophs were shown to be stronger affected by protozoan grazers.

211A Protein-SIP to elucidate bacterial key players in wetland ecosystems Jana Seifert[*1], Martin von Bergen[1], Paul Bodelier[2]. *[1]UFZ-Helmholtz Center for Environmental Research GmbH, Germany, [2]Netherlands Institute of Ecology (NIOO-KNAW), Netherlands*

Wetland ecosystems are major contributors to global methane emission to the atmosphere and therefore play an important role in our earth's climate. A large part of the methane produced in wetland systems is degraded before it can escape to the atmosphere. Methane-oxidizing bacteria (MOB) use methane carbon as substrate for energy generation as well as for the formation of cell biomass and thereby from an important regulating factor in methane cycling in wetlands. It has been demonstrated that known MOB species have different methane consuming capacities and react differently to environmental perturbation. Hence, in order to predict effects of environmental change on methane consumption it is important to know which MOB species or subgroups are active in the environment. To this end intact soil cores were retrieved from a plot along a riparian wetland representing a gradient of exposure to flooding with riverwater. The cores were incubated with 13C methane followed by protein-based stable isotope probing (protein-SIP) Functional metaproteomics as used in protein-SIP enables the detection of functional active species and allows an exact quantification of the heavy labeled isotope incorporation. In combination with metagenomics this functional metaproteomics approach can be used to reconstruct carbon and nitrogen fluxes in complex microbial communities. Features of protein-SIP are the high dynamic range of incorporation, the labeling ratio and the distribution of the isotopologue pattern. Protein extraction was done using 3g of freeze dried soil and a phenol extraction procedure. Protein extract were separated via 1-dimensional gel electrophoresis and peptides were recovered after in-gel tryptic digestion. Peptides were analysed by liquid chromatography-tandem mass spectrometry. Protein identifications showed a number of Methylobacter tundripaludum related proteins in all samples. Particulate methane monooxygenase subunit B (PmoB) was one of the prominent proteins revealing a relative isotope abundance of about 92%13C in the first three soil cores with a high flooding intensity. The detection of 13C incorporation only in type Ia methanotrophic bacteria showed that a minority in a great bacterial population are the key players of the methane oxidation process.

212A Bacterial population in the Pelagic zone Abhishek Srivastava[*1], Sarahi L. Garcia[2], Falk Warnecke[2], Hans-Peter Grossart[1]. *[1]Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Department [3]: Limnology of Stratified Lakes, Stechlin, Germany, [2]Jena School for Microbial Communication (JSMC) and Microbial Ecology Group at Friedrich Schiller University Jena, Germany*

Ultramicrobacteria represents the small-sized (<0.1 μm3) bacterial species of the fresh-water or marine bacterioplankton. They are also characterized by a limited genome size i.e. 0.58 to 3.2-Mb. They often numerically dominate the freshwater ecosystem and even account for more than 50% of entire bacterial population in the pelagic zone. With the advent of second-generation DNA sequencing, many projects have been initiated to get an insight of the ultramicrobacterial genomic information. Cultured bacteria *Polynucleobacter necessaries asymbioticus* belonging to the class betaproteobacteria was the first to be sequenced and offered great genetic details which remained obscure since long. Very recently, the genetic information of an uncultured *Actinobacteria* from ac1 lineage was revealed by individual cell sorting and subsequent DNA sequencing. This method bypasses the biggest problem of microbial-ecology where obtaining axenic and cultivable strain remains a bottleneck. Their genome comparison provides a glimpse of essential genes required for free-living life style in the epilimnetic strata of lake. Information interpreted from their genome sequence suggests that the timely gene expression for defense, repair, invasion, varied nutrient utilization and stress factors could be the key for their global success. And they not only seem to survive the unfavorable conditions, but they appear to be opportunistic due to the presence of genes which can cause cellular damage to other organisms possibly for self-protection or nutriments. Their genomic compendium also indicates various possibilities of symbiotic relationships.

213A A dark and winding road: from microbial sequences to soil biogeochemical fluxes in a global change experiment James T. Weedon*1, Rien Aerts1, George A. Kowalchuk2, Wilfred F.M. Röling3, Peter M. van Bodegom1 *1Department of Ecological Science, VU University Amsterdam, Netherlands, 2Department of Microbial Ecology, Netherlands Institute of Ecology, Wageningen, Netherlands, 3Department of Molecular Cell Physiology, VU University Amsterdam, Netherlands*

One of the core concepts underlying microbial biogeochemistry is that changes in the composition of the microbial community will lead to shifts in the production of enzymes involved in biochemical transformations, and consequently changes in the magnitude of biogeochemical fluxes. Or in other words, differences in biogeochemical fluxes are considered to be at least partly attributable to changes in the size and composition of the microbial communities driving them. However, in the highly diverse, spatiotemporally complex microbial communities that characterize the soil environment, such connections between community structure and ecosystem function (if they exist) have proven elusive. We present a summary of several recent studies in which we have investigated the relationship between experimental climate manipulation, microbial community structure, soil enzyme dynamics and carbon and nitrogen cycling in a sub-arctic peatland (Abisko, Sweden). Despite large effects of experimental summer warming on organic nitrogen turnover and peat respiration, we have found no clear effect of the treatment on the composition (community profiling with 16S rRNA gene) or function (potential activity and temperature sensitivity of soil enzymes) of the microbial community. Moreover, there was no relationship between any of these levels of organization. Hence, we need explicit conceptual and quantitative models that allow us to relate what we can measure (relative abundances of marker sequences, potential enzyme activities, net substrate accumulation) to what we actually want to know (identity and abundance of key microbial players, *in situ* enzymatic reaction rates, carbon and nutrient balances). We discuss which concepts might best be applied to achieve this.

Annex 2: List of participants

Signed hard copies will be transferred by surface mail.

EUROCORES Programme EuroEEFG

Copenhagen, 23-8-2012

ATTENDANCE LIST: ORAL CONTRIBUTED SESSION

***Meeting day*: 23-8-2012**

Nr	*Family Name*	*First Name*	*Country*	*Signature*	*Comments (official use only)*
1					
2	Stoeck	Thorsten	Germany		
3	Bertilsson	Stefan	Sweden		
4	Grossart	Hans-Peter	Germany		
5	Kowalchuk	George	Netherlands		
6	Urich	Tim	Austria		
7	Frenzel	Peter	Germany		
8	Bodelier	Paul	Netherlands		

Meeting Secretary (name and signature): ……

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1	Pachiadaki	Maria	Germany		
2	Krause	Sascha	Netherlands		
3	Seifert	Jana	Germany		
4	Henneberger	Ruth	Switserland		
5	Baltar	Frederico	Netherlands		
6	Neuenschwander	Stefan	Switserland		
7	Heinrich	Frederike	Sweden		
8	Liebner	Susanne	Norway		
9	Gadermaier	Maria	Austria		
10	Reim	Andreas	Germany		
11	Lüke	Claudia	Germany		
12	Bodelier	Paul	Netherlands		
13	Weedon	James	Netherlands		
14	Srivastava	Abishek	Germany		

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