

ESF Eurocores Ecological and Evolutionary Functional Genomics (EuroEEFG) workshop:

### **Understanding, managing and protecting microbial communities in aquatic and terrestrial ecosystems: “Exploring the trait-based functional biodiversity approach”.**

**Venue and dates:** Conference center ‘Hof van Wageningen’, 10-13 February 2013, Wageningen, the Netherlands.

**Proposer and organizer:** Paul L.E. Bodelier (PL, MECOMECON) and Sascha Krause (Postdoc, MECOMECON)

### **Summary**

It is generally recognized that human activities affect biological diversity and possible ecosystem functions on earth. However, with respect to microbes we have no idea to what extent human impact will affect diversity and functioning despite the fact that they are the crucial drivers of every biogeochemical cycle on this planet. This knowledge gap originates from the inherent methodological difficulties associated to studying microbes in natural environments but also stems from some misconceptions ecologists have on microbial diversity and functioning. The most important is the notion that microbial species are highly redundant. The functions they catalyze are inextinguishable and the high diversity and metabolic flexibility makes microbial communities insensitive to disturbance.

The advent of genomic and proteomic techniques offer the potential to monitor genes, transcripts, and proteins of microbial communities in high spatial as well as temporal resolution. However, current approaches to study the relationships of microbial diversity and ecosystem function (BEF) are focused on species richness of taxonomically described species. These approaches are limited by the definition of bacterial species which demarcates taxonomic units that can still be enormously diverse both in functionality and ecology. From plant sciences, a functional biodiversity approach using species, genus, or community functional traits may be a framework to be used in microbial BEF research. Descriptions of such trait-centered approaches and its incorporation into microbial process models have already been proposed.

This workshop aimed to bring together the EuroEEFG microbial oriented CRP’s (FREDI, MOCA, STRESSFLEA, and MECOMECON) to discuss trait-based approaches in microbial ecology. The 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. Together with four invited leading scientist in the field of trait-based microbial research this formed the perfect mixture to present and discuss CRP’s research in light of the general theme of the activity. The two day workshop enabled PhD students and post-docs to present their work within EuroEEFG and to set research corner marks for future projects. The interaction with well known scientist in related fields is a critically important experience which substantially contributes to the training and career building aspect of the program. In summary, this event represented a very efficient way and facilitated the networking and exchange of three CRP’s within EuroEEFG.

There was general consensus that trait-based approaches may expand our current understanding of microbial diversity and ecosystem function. However, it will be difficult to define and assess traits for microbial species since these are very much context dependent and may be highly plastic in the environment. It has been suggested that cultivation approaches, with individual or artificial mixtures of isolated microorganisms, are a first step towards a definition and assessment of traits in microbial species. Trait interactions can play a major role, however, to what extent this can be generalized in microorganisms needs to be investigated. In this context, the usually applied sampling strategies for environmental microbial communities are fundamentally different from higher animals and plants, i.e. in most cases it is not possible to sample a microbial community in the strict sense of the macro-ecological definition because multiple non-interacting communities are often bulked together. Hence, adopting concepts from macro-ecologists is a good start. However, we have to realize that we are studying microbial communities at different levels. Next to this, microbial systems may follow different rules which calls for an own body of concepts independent from known theory. Many of the oral contributions underlined the importance of microbial diversity and community composition for ecosystem function and their sensitivity against disturbances. It was concluded that it is time to line up all available examples in order to create awareness regarding the consequences of microbial biodiversity loss.

### **Final program of the event**

#### ***Sunday 10<sup>th</sup> February***

20.30-22.00: Get together in the hotel lobby

#### ***Monday 11<sup>th</sup> February***

8.45-9.00: **Paul Bodelier (NIOO) and Sascha Krause (NIOO)**

Welcome words.

9.00-10.00: **Laurent Philippot (UMR)**

Functional traits in microbial ecology: old wine in new bottles?

10.00-10.20: **Peter van Bodegom (VU Amsterdam)**

Analogies and concepts from macro-ecology to define challenges for traits based approaches in microbial ecology.

10.20-10.40: **Pascal Niklaus (University of Zurich)**

A plant sciences perspective on diversity and traits.

Break

11.00-12.00: **Elena Litchman (Michigan State University)**

Trait-based approaches to microbial ecology and evolution.

12:00-12:20: **Stefan Bertilsson (University of Uppsala)**

Functional diversity in the lake microbiome.

Lunch

14.00-14.20: **Anja Eggert (Leibnitz Institute for Baltic Sea Research)**

Simulating the response of the Benguela Upwelling System to future climate change: limitations of a traditional plankton ecosystem model.

14.20-14.50: **Hans-Peter Grossart (Leibnitz Institute for Freshwater Ecology and Inland Fishery)**

Ecophysiological adaptations of ultramicrobacteria: the key to their success.

Break

15.00-16.00: **Jay Lennon (Indiana University)**

Mapping the niche space of microorganisms using taxonomy and traits.

16.00-18.00: World cafe discussion groups

18.00-19.00: Poster session and drinks

19.00-21.00: Dinner

21.00-open: Networking activities

## Day 2 Tuesday 12<sup>th</sup> (February)

- 9.00-10.00: **Joana Falcão Salles (University of Groningen)**  
Does microbial diversity matter for ecosystem functioning?
- 10.00-10.20: **Xavier Le Roux (CNRS-INRA)**  
Functional traits of soil nitrite oxidizing bacteria explain their response to multifactorial global change.  
Break
- 10.40-11.00: **Paul Bodelier (NIOO)**  
The role of diversity and traits in methane cycling in wetlands: a model for conceptualizing microbial life strategies.
- 11.00-11.20: **Sascha Krause (NIOO)**  
Trait-based approaches in microbial ecology: A case study on methane oxidizing bacteria testing the phylogenetic signal of functional traits.
- 11.20-11.40: **Claudia Lüke (Max Planck Institute for Terrestrial Microbiology)**  
Macro-ecology of methane oxidizing bacteria: The  $\beta$ -diversity of pmoA genotypes in various environments.
- 11:40-12:30: Discussions  
Lunch
- 14.00-14.30: Summary and overview from discussion groups on day 1
- 14.30-16.30: World cafe discussion groups  
Break
- 17.00-18.00: General discussion and synthesis  
Closing dinner

## Day 3 (Wednesday 13<sup>th</sup> February)

- 8.30: Leave from Hotel
- 9.00-10:45: Tour through the NIOO building
- 11.00: Official end of workshop

## Poster session on Monday 11<sup>th</sup> February

### Ruth Henneberger (ETH Zurich):

Field-Scale Stable-Isotope Probing of active methanotrophs in a landfill-cover soil.

### Andreas Reim (Max Planck Institute for Terrestrial Microbiology):

Effect of energy flow on the susceptibility of aerobic methanotrophic communities to disturbance.

### Inga Richert (University of Uppsala):

Benzene uptake in a petroleum hydrocarbon contaminated environment - MAR-FISH to assess functional traits.

### Kristin Bergauer (University of Vienna):

A metaproteomic and -genomic assessment of microbial communities in the deep Atlantic Ocean.

### Caroline Souffreau (KU Leuven):

Influence of experimental mass-effects on bacterial community composition and C-source usage.

### Ellen Decaestecker (KU Leuven):

Host-symbiotic microbial community interaction and adaptation to cyanobacterial stress in the water flea *Daphnia*.

### Koen Rummens (KU Leuven):

Indoor rivalry, alien invasions or evolution? Importance of local, regional and evolutionary processes for bacterial community composition and function in shallow lakes.

### Martijn Kallens (KU Leuven):

Effect of *Microcystis* on the symbiotic bacterial community of *Daphnia*.

### **Scientific content of the workshop**

Ecosystems collectively determine biogeochemical processes that regulate the Earth System. Loss of biodiversity is generally regarded as detrimental to ecosystems and ecosystem functioning and therefore has been a central issue for environmental scientists during the last decades. Microorganisms (i.e., Bacteria, Archaea, protists, and fungi) comprise a major part of the total biomass of organisms and represent the largest source of biodiversity. They play critical roles in natural biogeochemical processes, sustaining ecosystem functioning. Many of the reactions catalyzed by microorganisms are fundamental to functioning of engineered or managed systems like wastewater treatment plants, agricultural fields, and industrial bioreactors providing many services to our societies. Considering the challenges we are facing with overexploitation of the planet, climate change, pandemics, increasing demands in food production and renewable energy, it is remarkable that microbes and their diversity are largely ignored in the ongoing debate on global biodiversity loss and conservations policy.

The research on biodiversity–ecosystem function (BEF) inherently requires the investigation of the relationship between species-assemblages and ecosystem processes. High diversity, rapid generation times, high adaptability due to genome rearrangements, and ubiquitous distribution, have led to the notion that microbial communities are highly redundant and omnipresent and therefore inextinguishable. However, the latter may be a misconception driven by a number of gaps in our assessment and understanding of the functioning of microbial communities and the relevance of microbial diversity in ecosystem functioning. These gaps in our knowledge are centered around diversity assessment, biogeographical distribution pattern, linking diversity to function, extent of functional redundancy, resistance, and resilience of microbial communities.

### ***From descriptive to predictive; from species richness to functional biodiversity***

It is obvious that the omission of microbial communities in management and conservation of ecosystems is due to a lack of understanding the functioning and composition of natural microbial communities leading to the assumption that microbial communities are insensitive, resilient, and redundant. However, in order to receive the attention of policy- and decision-makers, microbial diversity needs to be incorporated into predictive ecosystem process models. However, this will require a different approach as has been followed until now. Evidently, using richness of taxonomically described species is not the way to follow in microbial BEF research. Similar to plant–sciences, a functional biodiversity approach using species, genus, or community functional traits would make much more sense as a framework to be used in microbial BEF research, especially, where a clear species concept is still absent. Descriptions of such trait-centered approaches and its incorporation into microbial process models have been proposed. However, these models assume that functional traits and physiological responses are related to phylogeny and hence can be estimated from phylogenetic information only. The challenge remains, however, to establish basic relationships between traits, responses, and phylogeny.

High-throughput genomic and proteomic techniques offer the potential to monitor genes, transcripts, and proteins of microbial communities in spatial as well as temporal high resolution and thereby identify correlated microbial functional traits. However, to use these relationships in microbial BEF studies, we have to know the biology underlying these correlations and whether they can be used as ecosystem process proxies, possibly linked to phylogenetic categories. This information still has to be retrieved from experiments on cultures and from in situ studies.

The EuroEEFG platform offers a great range of participants interested in this concept. The **MOCA-FREDI-STRESSFLEA-MECOMECON CRP's, PhD and postdocs** investigate major microbial

habitats (marine, freshwater and terrestrial) as well as a focus ranging from broad scale functions (heterotrophy in freshwater) to functions carried out by very narrow phylogenetic groups (chemolithotrophs, methanotrophs). This consortium of projects harbors a multidisciplinary group of scientists ensuring a stimulating discussion to advance our understanding of natural microbial communities, their regulation mechanisms and function in a diverse terrestrial, aquatic system and man-made systems.

### ***General discussion***

The last part of the workshop was dedicated to a general discussion and the preparation of a possible joint publication. The first part of the discussion was centered on the statement that trait-based approaches are different from functional studies (e.g. relating gene copy numbers to function), which has already been done for quite some time in microbial ecology. It needs to be clearly pointed out that trait-based approaches are a new conceptual direction in microbial ecology. In this context, using traits can be only useful for microorganisms when the research question is clearly defined, e.g. basic ecological theory or prediction of ecosystem function. It would be a great benefit to develop a conceptual framework such as known for plant traits. But, there are difficulties with microbial systems, because there are many different microbial functions. In contrast, for plants “ecosystem functioning” this is generally assessed by looking at plant productivity, which is rather easy to measure. Nevertheless, traits can help to understand functioning, composition and dynamics in microbial communities.

It has been suggested to come up with an own conceptual framework which may also facilitate to explain unresolved patterns of higher animals and plants. To illustrate, for plants there is an occupation on carbon fluxes, but that is not the only function they perform, however, all classifications are based on that, hampering progress in plant ecology. For microorganisms this is different! There is such a versatility of functions which requires a lot of different approaches. Genetic information of microbes may be applicable as proxy of traits.

A central point of the final discussion underlined the importance of culture experiments. When evaluating the relevant traits for microbes we will not get around culture experiments and measuring traits and especially trait-response ranges in the lab. Beside the wealth of traits that are already available for 6-8000 species described in culture collections it will be necessary to measure traits using standardized protocols. From the existing data collections (e.g. ATCC, DSMZ, NCIMB, Biolog etc.), however, most of measured traits are not accessible in a database and require much manual work to be exploited.

To link organisms and their traits to ecosystem functions, it will be essential to evaluate the contribution of traits to the fitness of the organism. Next to this, in complex habitats it is impossible to sample and measure the same, species or community over time as it is done for plants and animals. Hence, extrapolation and adoption of existing macro-ecological theory may not be straightforward.

A final argument was related to the assessment of the importance of microbial diversity and microbial community composition for ecosystem functioning. To simplify predictions using traits-based approaches, it may already be sufficient to select the organisms/clusters of organisms that perform the rates that really matter (see examples le Roux on nitrification and Bodelier on methane oxidation). In that case, a plant functional type classification for a given function (separate for each function) instead of on phylogeny alone might be all that is needed. In that context, it is interesting that dilution experiments, removing the rare species, show there are effects on fluxes. Hence, the rare species matter.

There was a general consensus that the outcome of the discussions of the workshop would justify a publication that will highlight the importance of microbial diversity and traits for important ecosystem functions. A collection key-examples (nitrification, denitrification, methane oxidation, methanogenesis, freshwater and marine bacterioplankton) will show that there are many crucial functions carried out by specialized or adapted microbes that are not redundant which will put the known paradigms that are at the basis of excluding microbes from BEF debates and conservation issues.

### **Poster session**

On the first meeting day a poster session was organized to give young researchers the opportunity to present their work within the EuroEEFG framework. In total 8 posters have been presented. The abstracts of the posters are attached below in the annex of this report.

### **Results and impact on the EUROCORES program**

The workshop brought together a large group of scientists from different projects (MOCA, FREDI, STRESSFLEA, and MECOMECON) within the EuroEEFG program. Thereby, a large range of expertise were gathered, which covered not only major microbial habitats (marine, freshwater and terrestrial) but also broad scale functions (heterotrophy in freshwater), and functions carried out by very narrow phylogenetic groups (chemolithotrophs, methanotrophs). Hence, in combination with the invited speakers as leading scientists of trait-based research, this meeting formed a strong platform to discuss and push forward the application of a new way of assessing biodiversity of microorganisms. To disseminate the proposed ideas of the workshop, we anticipated publishing a research paper on the question why microbes are left out of the BEF debate and how trait-based approaches can help us to get microbial ecology at the level of plant- and animal ecology, or even beyond. We can put together convincing evidence that the paradigms of high redundancy, omnipresence and inability of extinction are definitely not general characteristics of microbial communities. The manuscript is in preparation and once published the dissemination of the EUROCORES program will be high.

Next to this, the complete workshop was monitored via the social media platform Twitter. 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 around the globe.

Besides the presentation of the science, 9 PhD students and post-docs within EuroEEFG had the opportunity to present and discuss their work, to interact with 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 oral presentation**

### **Functional traits in microbial ecology: old wine in new bottles?**

*Laurent Philippot, UMR 1347 Agroecology, Dijon, France*

Microbial communities have a central role in many ecosystem functions such as biogeochemical cycling, filtering and climate regulation. However, while functional trait-based approaches are commonly used for at least two decades in microbiology, most microbial ecology studies are still carried out with the rRNA genes. This talk will highlight the advantages of trait-based approaches for studying microbial diversity and its relationship with ecosystem functions. For this purpose, denitrification, a microbial process involved in N-cycling, was selected as a model functional trait. Using both naturally assembled and artificially manipulated communities, we will show how the diversity of the denitrifier community relates to process rates under optimal conditions but also under broad environmental gradients. We will also show that the analysis of spatial patterns of traits can help bridging microbial community ecology and ecosystem process in terrestrial environments.

### **Analogies and concepts from macro-ecology to define challenges for trait-based approaches in microbial ecology**

*Peter van Bodegom, Free University of Amsterdam, Department of Ecosystem studies, Amsterdam, the Netherlands*

Traits-based approaches are becoming increasingly popular within ecology, including microbial ecology. Two non-mutually exclusive applications of traits-based approaches include i) the description and understanding of species strategies and ii) to understand community assembly processes. While trait trade-offs have been used extensively in plant sciences to identify plant strategies, trait trade-offs are not yet well-understood to delineate strategies of micro-organisms except for distinguishing acquisitive vs. conservative strategies. While increased understanding of such trade-offs may aid predicting microbial mediated processes, this is currently hampered by lack of standardized trait measurements, potential culturing biases and lack of concepts on strategy axes. At the same time, the wealth of phylogeny information on microbial functional genes may help identifying proxies of traits when accounting for the strength of phylogenetic signals. The identification of microbial strategies is an important prerequisite for understanding microbial community assembly as it is one of the mechanisms leading to trait convergence under influence of environmental drivers. With the further quantification of microbial trait-environment relationships such community assembly processes may be more precisely defined, potentially allowing better predictions of microbial-mediated fluxes.

### **A plant sciences perspective on diversity and traits**

*Pascal Niklaus, University of Zurich, Institute of Evolutionary Biology and Environmental Studies, Zurich, Switzerland*

The application of trait-based approaches is well established in plant and animal science. In this presentation several approaches from plant ecology will be introduced. The focus will be on using existing concepts and theoretical frameworks for trait-based approaches centered on microorganisms.

### **Trait-based approaches to microbial ecology and evolution**

*Elena Litchman, Michigan State University, W.K. Kellogg Biological Station, Department of Zoology and Ecology, Evolutionary Biology and Behavior Program, MI, USA*

Trait-based approaches hold the potential to provide a more mechanistic framework for explaining and predicting the structure and dynamics of ecological communities. Here I present examples how trait-based approaches can be used to understand microbial (phytoplankton) community structure along various environmental gradients. Traits often are inter-correlated and these correlations may represent trade-offs arising from the fundamental surface area to volume relationships or enzyme kinetics. Different functional groups of phytoplankton appear to be clustered along some trade-off curves. This clustering corresponds to distinct ecological strategies. Traits may exhibit large scale biogeographical

patterns, for example, temperature optima for growth in marine phytoplankton showed a highly significant latitudinal gradient that could be explained by local adaptation. In summary, traits and trade-offs of microbes can help explain and predict current and future distributions and community structure of microbial communities.

### **Functional diversity in the lake microbiome**

*Prof. Dr. Stefan Bertilsson, University of Uppsala, Evolutionary Biology Centre, EBC, Department of Ecology and Genetics, Uppsala, 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 is 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.

### **Simulating the response of the Benguela Upwelling System to future climate change: limitations of a traditional plankton ecosystem model**

*Anja Eggert, Leibniz-Institute for Baltic Sea Research, Rostock, Germany*

Within the GENUS-project (Geochemistry and Ecology of the Namibian Upwelling System) funded by the German Federal Ministry of Education and Research (BMBF, reference number 03F0497A) we develop a regional 3-dimensional ecosystem model, designed to simulate the specific nutrient, oxygen and sulfur dynamics on the Namibian shelf in the water column and in the sediment. Strong upwelling is driven by the southern trade winds supply cold, nutrient rich water. This supports high primary production resulting in a large flux of sinking detritus. The biological oxygen demand is high and additionally, nutrient-rich but oxygen-depleted water is advected from the North. Hence, hypoxic conditions on the Namibian shelf are wide spread. Notably high rates of sulfate reduction in the sediment generate high concentrations of dissolved hydrogen sulfide in the surface sediment layers and may be released intermittently to the water column. Large chemoautotrophic sulfur bacteria thrive on hydrogen sulfide and form conspicuous mats on the sediment. Denitrification and nitrification are important components of the nitrogen cycle and anaerobic ammonium oxidation is known to play a significant role as nitrogen sink in the Benguela upwelling system. Organisms at higher trophic levels like zooplankton play an important role for mineralization but also for the vertical and lateral transport



of organic matter. The physical model component is MOM-4 (Geophysical Fluid Dynamics Laboratory, GFDL). The ecosystem model is a NPZD-model (Nutrients-Phytoplankton-Zooplankton-Detritus). Three phytoplankton and three zooplankton functional types are distinguished. Ecologically relevant processes mediated by prokaryotes in this ecosystem are implemented and the environmental conditions (e.g. oxygen concentration, temperature etc.) define the metabolic rates. One aim of the modeling activities within the GENUS-project is to simulate ecosystem response to future climate change. We will use climate scenarios of a regional atmospheric model to force the coupled hydrodynamic-ecosystem model. However, one major drawback of plankton functional type-modeling is its structural rigidity. Structure and parameterization are defined in the first place and therefore are not adequate to simulate ecosystem responses to past or future climate change. Therefore a trait-based modeling approach may be applicable in future studies.

### **Ecophysiological adaptations of ultramicrobacteria: the key to their success**

*Hans-Peter Grossart, Leibniz-Institute of Freshwater Ecology and Inland Fishery, Experimental Limnology, Berlin, Germany*

Ultramicrobacteria represents the small-sized (<0.1  $\mu\text{m}^3$ ) 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 necessarius 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 nutrients. Their genomic compendium also indicates various possibilities of symbiotic relationships.

### **Mapping the niche space of microorganisms using taxonomy and traits**

*Jay T. Lennon, Indiana University, Department of Biology, IN, USA*

The biodiversity of microbial communities has important implications for the stability and functioning of ecosystem processes. Yet, very little is known about the environmental factors that define the microbial niche and how this influences the composition and activity of microbial communities. In this talk, I describe a study where we derived niche parameters from physiological response curves that quantified microbial respiration for a diverse collection of soil bacteria and fungi along a soil moisture gradient. Within their limits of activity, microorganisms exhibited a wide range of responses, suggesting that some taxa may be able to coexist by partitioning the moisture niche axis. For example, we identified dry-adapted generalists that tolerated a broad range of water potentials, along with wet-adapted specialists with metabolism restricted to less-negative water potentials. These contrasting ecological strategies had a phylogenetic signal at a coarse taxonomic level (phylum), suggesting that the moisture niche of soil microorganisms is highly conserved. In addition, variation in microbial responses along the moisture gradient was linked to the distribution of several functional traits. In particular, strains that were capable of producing biofilms had drier moisture optima and wider niche breadths. However, biofilm production appeared to come at a cost that was reflected in a prolonged lag

time prior to exponential growth, suggesting that there is a trade-off associated with traits that allow microorganisms to contend with moisture stress. Together, we have identified functional groups of microorganisms that will help predict the structure and functioning of microbial communities under contrasting soil moisture regimes.

### **Does microbial diversity matter for ecosystem functioning?**

*Dr. Joana Falcão Salles, University of Groningen, Center for Life Sciences, Department of Microbial Ecology, Groningen, the Netherlands*

Research on biodiversity and ecosystem functioning takes many angles, from the diversity and stability of communities to the preservation of ecosystems and ecosystem services. In spite of the overwhelming microbial diversity, the concern about how environmental changes affect life in our planet has also taken microscopic scales. It is therefore crucial to understand how ecosystem processes are affected by microbial diversity. Here, several examples will be discussed to find an answer to the question if microbial diversity really matters for ecosystem function.

### **Functional traits of soil nitrite oxidizing bacteria explain their response to multifactorial global change**

*Xavier Le Roux, University of Lyon-Villeurbanne, CNRS-INRA, Microbial Ecology Laboratory, Villeurbanne, France*

Responses of soil nitrogen (N) cycling to simultaneous and potentially interacting global environmental changes are uncertain. Here, we investigated the effects of several global change factors and their interaction on the activity, abundance and diversity of the soil nitrite-oxidizing bacteria (NOB). In addition, we tested whether known functional traits of NOB can explain their response to global change scenarios. This work is part of the Jasper Ridge Global Change Experiment (CA, USA). This field experiment included four treatments CO<sub>2</sub>, temperature, precipitation, nitrogen with two levels per treatment (ambient and elevated), and all their factorial combinations replicated six times. Among soil NOB, *Nitrospira* are K strategists adapted to low N availability, whereas *Nitrobacter* are r strategists adapted to higher N levels. Among *Nitrobacter*, some are chemolithotrophs whereas others have mixotrophic capacity (e.g. *N. hamburgensis*). Hence, two main trait types can thus be considered for NOB: r versus K strategy as regards to N; and ability to use organic carbon. We collected soil samples of treatments, and measured potential nitrite oxidation, *Nitrobacter*- and *Nitrospira*-like NOB abundance (qPCR), dominant populations of *Nitrobacter*-like NOB (cloning-sequencing), and ancillary environmental variables (respiration, soil water content, N immobilization and nitrification, gross rates of N mineralization). Results demonstrated that changes in nitrite oxidation in soil is mainly driven by *Nitrobacter*-like NOB abundance under all treatments, and both increased for global change scenarios which increase N availability (whereas *Nitrospira* tend to respond negatively). We identified a shift from autotrophic NOB driven by N availability to mixotrophic NOB that also depend on organic C availability which is induced when multiple global changes factors were applied.

### **The Role of Diversity and Traits in Methane Cycling in Wetlands: a model for conceptualizing microbial life strategies**

*Paul L.E. Bodelier, Netherlands Institute of Ecology, Wageningen, the Netherlands*

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. Although microbial processes are fundamental to methane emission from wetlands, the diversity of microbial communities and traits of the microbes involved are not taking into consideration in assessing potential sources of variation in the global methane budget. Production of methane by methanogenic archaea in wetlands is a major source while consumption by methane oxidizing bacteria in these ecosystems is a major sink. There are many environmental factors that control these processes, which have also been extensively investigated. The advent of a multitude of

culture independent techniques has resulted in a glimpse of the possible role of microbial community composition and or response in methane cycling reactions. Especially, the combined use of molecular biological techniques with the application of stable isotopes has led to valuable insight into the link between microbial characteristics and biogeochemical processes. Stable isotope probing studies targeting RNA, DNA, lipids and proteins demonstrate that in ecosystems important for global methane cycling only a limited number of species is responsible for production and consumption of methane. The limited functional-diversity combined with the tracking of the active species enables the assignment of life strategies to methane processing bacteria, conceptualizing their traits and ecological characteristics. We will propose a life-strategy concept which may have application for microbial ecology in general.

### **Trait-based approaches in microbial ecology: A case study on methane oxidizing bacteria testing the phylogenetic signal on functional traits**

*Sascha Krause\**, *Peter van Bodegom*, *Paul L.E. Bodelier*. \**Netherlands Institute of Ecology, 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, which is the statistical dependence among species traits through their phylogenetic relationship. We applied methods well established in evolutionary biology. We applied the software package Picante, Ape, and Phangorn 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 physiological (optimal growth temperature) and biochemical (GC content) traits. For the functional traits of MOB, there was a phylogenetic signal detected for the methane oxidation kinetics (that is  $K_m$ ) 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.

### **Macroecology of methane oxidizing bacteria: The $\beta$ -diversity of *pmoA* genotypes in tropical and subtropical rice paddies**

*Claudia Lüke\*, Peter Frenzel, Adrian Ho, Dian Fiantis, Peter Schad, Bellinda Schneider, Lorenz Schwark, and Sri Rahayu Utami, \*MPI for Terrestrial Microbiology, Marburg, Germany*

Even though studies addressing microbial biogeography have increased during the past decade, research on microbial dispersal is still in its infancies and many aspects are only poorly understood. Here, we compared the methanotroph community in paddy soils sampled in Indonesia, Vietnam, China and Italy, focusing on the distance-decay relationship that is well known from the biogeography of macro-organisms. We used the *pmoA* gene as marker for methanotroph diversity in T-RFLP, microarray and pyrosequencing approaches. We could observe a significant increase of  $\beta$ -diversity with geographical distance at the global (12,000 km) and regional scale (20 km). Measured environmental parameters explained only a small amount of data variation and we found no evidence for dispersal limitation. Thus, we propose historical contingencies being responsible for the observed patterns. Furthermore, we performed an in-depth analysis of type II methanotroph *pmoA* distribution at the sequence level. We used ordination analysis to project sequence dissimilarities into a 3-dimensional space (multidimensional scaling). The ordination suggests that type II methanotrophs in paddy fields can be divided into five major groups, however, their diversity is widely distributed independent of the geographic origin. No distance-decay relationship was observed at sequence level. By including tropical field sites (Indonesia and Vietnam) into the analysis, we further observed the first paddy fields harboring a methanotroph community depleted in type II methanotrophs.

### **Annex 2: Abstracts of poster session**

#### **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 (CH<sub>4</sub>) is a potent greenhouse gas contributing strongly to global climate change. Aerobic methane-oxidizing bacteria (MOB) play an important role in mitigating CH<sub>4</sub> emission to the atmosphere by utilizing CH<sub>4</sub> as sole energy and carbon source. MOB are ubiquitous in soils and particularly abundant in CH<sub>4</sub>-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, <sup>13</sup>CH<sub>4</sub> 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 CH<sub>4</sub> oxidation rates ranging from 1.8 to 58.2 mmol CH<sub>4</sub> (L soil air)<sup>-1</sup> d<sup>-1</sup>. Significant lateral variation in activity was observed, and oxidation rates were positively correlated with mean CH<sub>4</sub> 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 CH<sub>4</sub> 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  $^{13}\text{C}$  into characteristic MOB fatty acids (FAs) was clearly demonstrated by increased  $\delta^{13}\text{C}$  values (up to  $\sim 1500$  ‰) compared to natural background values. The amount of  $^{13}\text{C}$  incorporation into biomass was positively correlated with  $\text{CH}_4$  oxidation rates. In general, FAs  $\text{C}_{14}:0$ ,  $\text{C}_{16}:1\omega 8$ ,  $\text{C}_{16}:1\omega 7$  and  $\text{C}_{16}:1\omega 6$  (type I MOB) showed highest label incorporation, while FAs  $\text{C}_{18}:1\omega 8$  and  $\text{C}_{18}:1\omega 7$  (type II MOB) were only labeled 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  $\text{CH}_4$ , while the results also indicate that MOB abundance and community structure are driving factors in  $\text{CH}_4$  oxidation at this landfill.

### **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 methanotrophic 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 soil into a  $\gamma$ -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 methanotrophic 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.

### **Benzene uptake in a petroleum hydrocarbon contaminated environment - MAR-FISH to assess functional traits**

*Inga Richert*, *University of Uppsala, Evolutionary Biology Centre, EBC, Department of Ecology and Genetics, Uppsala, Sweden*

In events of petroleum spill into aquatic environments, petroleum hydrocarbons stay recalcitrant for many decades. High compound load combined with its low availability, fast oxygen depletion, toxicity and nutrient limitation lead to the formation of specialized microbial communities processing the complex hydrocarbons, as aliphatic, mono- and poly-aromatic hydrocarbons, at very low activities. Sequencing approaches identified ecotypes, which typically occur in petroleum-contaminated environments; nevertheless their individual ecological role in the environment remains unclear. Anaerobic benzene degrading populations, taken from the environment, serve as a model system for identification of key players in the mono-aromatic benzene break down and incorporation. Using microscopic based fluorescence *in situ* hybridization combined with micro-autoradiography (MAR-

CARD FISH) after incubation with  $^{14}\text{C}$ -labeled benzene, allow a robust *in situ* characterization of substrate utilization performed by specifically targeted phylogenetic groups. We aim to discuss the effect of environmental changes to native microbial populations; a question we therefore address is how the composition and benzene-incorporation is shifting after introducing manipulation in form of water table fluctuations. Water table fluctuations are expected to result in a stimulation of benzene incorporation due to increased distribution of nutrient and oxygen, but also due to re-oxidation of alternative electron acceptors. But more interesting will be the effect on the community structure, revealing if the manipulation causes a significant shift in diversity and composition. The probe set for CARD FISH and the protocol for micro-autoradiography are established, but concrete results are still to be expected.

### **A metaproteomic and -genomic assessment of microbial communities in the deep Atlantic Ocean**

*Kristin Bergauer\**, *Juan Antonia Garcia*, *Richard Sprenger*, *Gerhard J. Herndl*, *\*Dept. of Marine Biology, Faculty Center of Ecology, University of Vienna, Vienna, Austria*

It is well known that deep-water microbes mediate a substantial fraction of biogeochemical processes in the pelagic realm of the ocean with yet unknown metabolic pathways. Evidence has been presented that dark  $\text{CO}_2$  fixation does not only occur in the suboxic and anoxic water bodies but also in the oxygenated meso- and bathypelagic waters (200m – 4000m) of the open ocean. In these waters, dark  $\text{CO}_2$  fixation rates were reported to be similar to heterotrophic prokaryotic biomass production. Archaeal nitrification, which has been assumed to be the main energy source for inorganic carbon fixation, might be insufficient to supply fixation rates measured in some oceanic provinces of the North Atlantic. Hence, microbial lineages and energy sources responsible for carbon fixation in the dark ocean remain to be discovered.

### **Influence of experimental mass-effects on bacterial community composition and C-source usage**

*Caroline Souffreau*, *KU Leuven, Laboratory of Aquatic Ecology and Evolutionary Biology, Leuven, Belgium*

I am currently a postdoc at the Lab of Aquatic Ecology, Evolution and Conservation (KU Leuven) working on bacterial metacommunities. I study the relative importance of ecological processes structuring these communities (lineage sorting, dispersal limitation, mass effects and priority effects) and use freshwater bacterioplankton of shallow lakes as study system. At the local level, I try to connect the bacterial community structure both with biotic variables (phytoplankton, zooplankton, fish communities) and abiotic parameters (nutrient concentrations, etc). Next to ecological processes, I now start to study the importance of evolution in shaping bacterial communities in parallel to ecological processes. I therefore use *Pseudomonas*, and will link population genetics and geographical population structure of *Pseudomonas* species with functional traits at the species level (evolution) and community level (ecological processes). I am affiliated with EuroEEFG through the STRESSFLEA project.

### **Host-symbiotic microbial community interaction and adaptation to cyanobacterial stress in the water flea *Daphnia***

*Ellen Decaestecker*, *KU Leuven, Laboratory of Aquatic Ecology and Evolutionary Biology, Leuven, Belgium*

The wide array of genotypes presented by the symbiotic microbial community (SMC) and their ability to adapt rapidly to novel conditions combined with host control over the microbiota could help to maximize host fitness. Further, the nature and outcome of these symbiotic interactions are often context-dependent. Using exposure to toxic cyanobacteria as key environmental stressor in the water flea *Daphnia*, we here investigated the interaction between the host and the SMC. We therefore exposed axenic *Daphnia* to a diverse naturally occurring microbial community and to toxic cyanobacteria. We further aim to investigate what the impact is of the SMC and stress-induced

changes in the SMC on host genotype interactions. It is currently not known how environmental stress affects symbiotic microbiota and host-SMC interactions or what the consequences of SMCs are for adaptation in natural ecosystems. If our study reveals strong associations between SMCs and the investigated stressors, it may change evolutionary paradigms, in the sense that the unit of selection may not be the host genome only.

### **Indoor rivalry, alien invasions or evolution? Importance of local, regional and evolutionary processes for bacterial community composition and function in shallow lakes**

*Koen Rummens\*, Caroline Souffreau, Luc De Meester, \*KU Leuven, Ecology, Evolution and Biodiversity, Conservation Section, Leuven, Belgium*

Microbial communities play a key role in many crucial ecosystem processes. Yet, the processes determining the species composition of natural bacterial communities are insufficiently known. Local (lineage sorting, i.e. local selection by (a)biotic factors) as well as regional (dispersal) and evolutionary dynamics might all play an important role in determining bacterial community composition, but the relative importance of, and interactions between these dynamics remain largely unknown. Also the (indirect) effect these dynamics have on the functional characteristics of bacterial communities isn't clear. During this research we will quantify the relative importance of lineage sorting, mass effects, priority effects and evolution for the structuring of bacterial communities and investigate the impact of differential community composition on functionality. For this purpose, natural planktonic and biofilm communities from different pond types will be manipulated and cross-inoculated in specific experimental setups, including different immigration histories to test for the importance of priority effects. We will also characterize functional characteristics of lineages through time so that we will be able to answer questions like "Are priority effects occurring due to a numerical advantage or mediated by evolution and genetic adaptation of the first colonist?". By using natural communities sampled from shallow lakes we aim at gaining insight in these processes in complex, species rich systems.

### **Effect of *Microcystis* on the symbiotic bacterial community of *Daphnia***

*Martijn Kallens, KU Leuven Kulak, Laboratory of Aquatic Biology, Department of Biology, Kortrijk Belgium*

The animal body forms a habitat for a diverse and dynamic symbiotic microbial community (SMC), which has an influence on the health and fitness of its host. Environmental factors can alter the composition of this SMC, which in turn can affect its functionality. We conducted an experiment to investigate the effect of the toxic cyanobacterium *Microcystis* on the composition of the SMC in *Daphnia*. We also aim at investigating the functional role of the SMC for *Microcystis* tolerance in *Daphnia*.

## Annex 3: List of participants

Signed hard copies will be transferred by surface mail.



**EUROCORES Programme**  
European Collaborative Research

### ATTENDANCE LIST:

Meeting day: 11-2-2013 and 12-2-2013

<i>Nr</i>	<i>Family Name</i>	<i>First Name</i>	<i>Country</i>	<i>Signature</i>	<i>Comments</i>
1	Krause	Sascha	Netherlands		
2	Bodelier	Paul	Netherlands		
3	Henneberger	Ruth	Switzerland		
4	Bertilsson	Stefan	Sweden		
5	Richert	Inga	Sweden		
6	Van Bodegom	Peter	Netherlands		
7	Souffreau	Caroline	Belgium		
8	Lueke	Claudia	Germany		
9	Reim	Andreas	Germany		
10	Niklaus	Pascal	Switzerland		
11	Grossart	Hans Peter	Germany		
12	Rudnick	Max	Netherlands		
13	Bergauer	Kristin	Austria		
14	Decaestecker	Ellen	Belgium		
15	Callens	Martijn	Belgium		
16	Rummens	Koen	Belgium		
17	Eggert	Anja	Germany		
18	Le Roux	Xavier	France		
19	Philippot	Laurent	France		
20	Lennon	Jay T	USA		
21	Litchman	Elena	USA		
22	Falcao Salles	Joana	Netherlands		

Workshop coordinator (name and signature): .....