PS11 – Archaea: Important players in diverse microbial ecosystems

001B Methanogenic and methanotrophic archaeon communities in Antarctic permafrost samples differed in biogenic methane content

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The objective was to study archaeal diversity in biogenic-methane differing Antarctic permafrost core layers collected near Russian Bellingshausen station (King-George Island) using 16S rRNA gene sequencing. The hypothesis was that high methane content soil layer consists of active methanogenic archaeal communities while in low methane content layer methanotrophy both archaeal and bacterial dominates.

The 10-m deep borehole was drilled at I marine terrace 15m above sea level with mean annual air temperature (MAAT) -2°C. According to AMS radiocarbon dating of marine sediments this terrace have been formed about 7500 years ago what is in agreement with history of this area, where deglaciation begun about 6-9 kyr ago with climatic optimum occurred between ca. 4000 and 3000 year BP. Two soil layer samples featured by high and low biogenic methane content: No. 6 (615-625cm horizon, mostly sand, methane 7.4 ml/kg) and No. 12 (965-975cm horizon, mostly blue mica, methane 1.7 ml/kg). The soil core layers temperature averaged -1.5C.

The clone libraries constructed using two different size 16S rRNA gene PCR amplicons showed rather low abundance of archaeon phylotypes. Total eight Archaea phylotypes were recovered in both samples. Of them, three phylotypes were assigned (by >98% similarity in a sequence) to Methanobacteriales (Methanobacterium oryzae), Methanosarcinales (Methanosarcina semesiae) and Methanomicrobiales (Methanogenium boonei) while 5 others were left unidentified (≤91% similarity with known taxa). Only one (unidentified) phylotype was shared by both soil layer clone library. At the same time all the phylotypes recovered proved to be con-specific to environmental unidentified clones or isolates recorded in methane seep sediments representing both methanogenic and methanotrophic archaea (ANME) along with members of the Deep-Sea Archeal Group (DSAG).

As a preliminary result in both permafrost layers differing in methane content both methanogenesis and methanotrophy are microbially feasible. Nevertheless, higher methane content in 6m deep sample could be due to dominated methanogens of Methanosarcinales and Methanobacteriales while in less methane containing deeper sample methane consumption seems to be prevailing thanks to more abundant ANME archaeal phylotypes and practical absence of identified methanogens.

389B Temporal patterns in the abundance and diversity of ammonia oxidizing archaea and bacteria in aquarium biofilters

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Nitrifying biofilters in aquaria and aquaculture systems are generally augmented with ammonia oxidizing bacteria (AOB) to enhance start-up and ensure low ammonium levels. However, ammonia oxidizing archaea (AOA) were recently identified as the dominant ammonia oxidizers in freshwater aquaria, which raises questions about the appropriateness of the augmentation practice. In this study, we investigated aquarium filter biofilms to assess AOA and AOB abundance and diversity over time, and to identify possible impacts of maintenance and cleaning events on the nitrogen balance. Over a period of four months, ammonia oxidizing communities were investigated at four or five time points in each of seven aquaria (six freshwater; one marine). Nitrogen balances were made in three of the freshwater aquaria, showing biofilter loading rates of 0.27-0.32 mg N L⁻¹ d⁻¹ and complete nitrification (NH₄⁺ to NO₃⁻ without NO₂⁻ accumulation) represented the dominant nitrogen cycle pathway. Quantitative PCR (qPCR) data from bacterial and thaumarchaeal ammonia monoxygenase (amoA)
genes showed that AOA were numerically dominant in all freshwater biofilters. Moreover, in four of the six biofilters, AOA contributed all detectable amoA genes. In contrast, in the marine aquarium, AOB outnumbered AOA by 3 to 5 orders of magnitude based on amoA gene abundances. Denaturing gradient gel electrophoresis (DGGE) fingerprints of thaumarchaeal 16S rRNA genes revealed stable community composition within most individual aquaria over the studied period. DGGE patterns were also highly similar between different freshwater aquaria, with many bands shared. In contrast, fingerprints obtained from the marine aquarium were distinct from the freshwater aquaria, and nonmetric multidimensional scaling (NMDS) indicated separation of freshwater and marine fingerprints. This study revealed temporal stability of ammonia-oxidizing communities in aquarium biofilters, dominated by similar AOA communities in freshwater systems, and hence no impact of maintenance and cleaning events.

The distribution and ecophysiology of novel thermophilic, non-ammonia-oxidizing thaumarchaeia in Yellowstone National Park
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The newly described candidate archaeal phylum, Thaumarchaeota, presently consists of autotrophic, ammonia-oxidizing organisms. Recent studies have suggested the possible importance of ammonia-oxidation in high-temperature environments based on the presence and activity of ammonia monoxygenase genes (subunit A), and the isolation of two thermophilic ammonia-oxidizing thaumarchaeia. However, the distribution of thermophilic thaumarchaeia in Yellowstone National Park, and the origin of ammonia monoxygenase genes in geothermal environments are unknown and other metabolisms have yet to be explored in members of this phylum. Therefore, the objectives of the current study were to determine the distribution and ecophysiology of novel thaumarchaeia in geothermal environments, with emphasis on long-term study sites in Yellowstone National Park. A multidisciplinary approach was utilized including geochemical, metagenomic, molecular, and phylogenetic analyses. The pH and temperature range of the iron oxide and elemental sulfur geothermal springs sampled were 2.4-6.5 and 44-85˚ C, respectively. Thaumarchaeal-like 16S rRNA genes are widely distributed in these geothermal environments and occasionally comprise up to 84 % of the total archaeal 16S rRNA gene sequences. Phylogenetic analysis using the 16S rRNA gene predicts that these novel thaumarchaeal sequences branch deeper than any currently known representative of this phylum. These new thaumarchaeal 16S rRNA gene sequences represent three new phylogenetic clades within the Thaumarchaeota: sequences from iron oxide mats (Group I.1d), sequences from both iron oxide and elemental sulfur sediments (Group I.1e), and the most deeply-rooted sequences were exclusively from higher pH (5-6), sub-oxic sulfur sediments (Group I.1f). Thaumarchaeal-like de novo assemblies were obtained from iron oxide mat (Beowulf Spring) and elemental sulfur sediment communities (Dragon Spring) using nucleotide word frequency-principal components analysis. A significant fraction of the assembled metagenome sequence from Beowulf (21 %) and Dragon (~11 %) were contained within a single taxon exhibiting consistent sequence character. A total of ~1.5 Mb of thaumarchaeal-like sequence was identified from both Beowulf and Dragon Springs with average G+C contents of 45.6 ± 2.0 and 40.3 ± 2.0, respectively. Phylogeny of topoisomerase IB proteins and other phylogenetic marker proteins confirmed the deeply-rooted nature of these novel thaumarchaeia. Ammonia monoxygenase subunit A genes could not be identified in 25 metagenomes or amplified from various geothermal springs in Yellowstone National Park and are not found in de novo assemblies. Both assemblies suggest utilization of oxygen as a terminal electron acceptor as well as nitrate (Beowulf) or sulfur (Dragon). Heterotrophic growth of these thaumarchaeotaes is possible via the oxidation of simple or complex organic compounds. The thaumarchaeon from Beowulf Spring also has the potential to grow lithotrophically using hydrogen sulfide as an electron donor. Our results show that novel thermophilic thaumarchaeia are distributed across a wide variety of geothermal springs in Yellowstone National Park. They are also phylogenetically distinct from their mesophilic and thermophilic relatives. Most importantly, these thaumarchaeia contain energy-yielding pathways other than ammonia oxidation, which has yet to be described for this phylum. This study has expanded the known diversity and metabolic capabilities of high-temperature representatives of the candidate phylum, Thaumarchaeota.
003B Archaeal abundance across a pH gradient in an arable soil and its relationship with bacterial and fungal growth rates
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Soil pH is one of the most influential factors for the composition of bacterial and fungal communities, but the influence of soil pH on the distribution and composition of soil archaeal communities has yet to be systematically addressed. The primary aim of this study was to determine how total archaeal abundance (qPCR based estimates of 16S rRNA gene copy numbers) is related to soil pH across a pH gradient (pH 4.0-8.3). Secondarily, we wanted to assess how archaeal abundance related to bacterial and fungal growth rates across the same pH gradient. Bacterial growth was estimated using the leucine and thymidine incorporation technique and the acetate-into-ergosterol-incorporation method was used to estimate fungal growth.

We identified two distinct and opposite effects of pH on the archaeal abundance. In the lowest pH range (pH 4.0-4.7) the abundance of archaea did not seem to respond to pH. Above this pH range and up to pH 5.1-5.6 there was a sharp, almost 4-fold, decrease in archaeal abundance. The low archaeal abundance at pH 5.1-5.6 then sharply increased almost 150-fold with pH, resulting in an increase in the ratio between archaeal and bacterial copy numbers from 0.002 at pH 5.2 to more than 0.07 at pH 8. Furthermore, in the high pH range (pH 5.1-8.3) we also identified a strong negative relationship between fungal growth and archaeal abundance, and above pH 6.0, a strong negative relationship between specific bacterial growth rate and archaeal abundance.

Our results show that soil pH is an important factor that regulates total archaeal abundance in terrestrial environment. The non-uniform archaeal response to pH could be a reflection of variations in the archaeal community composition along the gradient, with some archaea being adapted to acidic conditions, and others to neutral to slightly alkaline conditions. This suggestion is reinforced by observations of contrasting outcomes of the (competitive) interactions between archaea, bacteria and fungi towards the lower and higher ends of the examined pH gradient.

004B Abundance and diversity of Ammonia-oxidizing Archaea and Bacteria in Lake Superior and Erie
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Nitrification, one of the major steps in the global nitrogen cycle, is a two-step process with the ammonia oxidation being the first and rate-limiting step. Different groups of microorganisms – Ammonia-oxidizing Archaea (AOA) and Bacteria (AOB) – are involved in ammonia oxidation. Lake Superior and Erie are part of the Great Lakes system, the largest group of freshwater lakes in the world. The two lakes differ in trophic status with Lake Superior being oligotrophic and Erie mesotrophic. This project aims to characterize the abundance and diversity of AOA and AOB in sediment samples from those lakes.

In October 2010 sediment samples were collected from both lakes (4 from Lake Erie and 8 from Lake Superior). Primers targeting the amoA (ammonia monoxygenase) gene were used to characterize abundance and diversity of both groups. The abundance (copy number) was determined with qPCR. Diversity was accessed by a pyro-sequencing approach utilizing six bar coded primers per run. The raw sequencing data were binned, quality filtered using QIIME with standard parameters, translated into protein sequences and filtered to exclude sequences with stop codons and frame shifts. The resulting sequences were aligned and used to determine the phylogeny and diversity. Abundance and diversity was linked to environmental parameters of the lakes.

Copy numbers of archael and bacterialamoA genes were in the same order of magnitude in Lake Erie, while in Lake Superior up to 4 orders of magnitude more archael than bacterialamoA copies were detected. Pyro-sequencing was conducted for the AOA in all samples and for AOB only in the samples from Lake Erie because the number of AOB was very low in the samples from Lake Superior. The AOB detected in the samples from Lake Erie belonged to theNitrosomonas oligotropha cluster, theNitrosomonas communis cluster and theNitrospira cluster, all groups of AOB that are frequently detected in freshwater. Clear differences were detected between the phylogenetic affiliations of the
AOA from the two lakes. Most sequences detected in Lake Erie clustered in the Thaumarchaeal soil group I.1b, where as most of the sequences in Lake Superior were found in the Thaumarchaeal marine group I.1a. Interestingly the number of phylotypes (alpha diversity) of the AOA was lower in the samples from Lake Superior than in Lake Erie.

Our results show that the populations of AOA and AOB in Lake Erie and Superior differ in abundance and diversity. These differences are very likely related to the different trophic states of the lakes.

005B Archaeal community structure and diversity of Charcoal from Amazonian dark earth
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Microorganisms that belong to the domain Archaea have been presenting an important role in the N and C cycles. The study of archaea in soils is relatively recent, therefore, its structure and diversity, as well as the factors that control this diversity are still not well understood. The areas of Amazonian Dark Earth (ADE) present high levels of P, Ca, pH and low Al saturation. The stability of organic matter in these areas can be three times higher in comparison to their original or background soil. This factor is probably due to the mineralogical characteristics of these organic soils, or due to the high amount of charcoal, which may reach seventy times more of this material, than their background or adjacent soil (ADJ). Charcoal may serve as habitat to the survival of microorganisms and protection against abiotic stresses. The aim of this study was to evaluate archaeal communities present in pyrogenic charcoal fragments from ADE soils from ADE sites. Therefore, soil samples were collected from four ADE archaeological sites in the Central Amazonia. The assessments of these communities were based on three different methodologies, which evaluated the archaeal 16S rRNA gene: fingerprinting based on T-RFLP and DGGE analyses to evaluate the structure of archaeal communities in this soils; and high-throughput sequencing (pyrosequencing) to estimate the abundance and assess the archaeal community composition. The results obtained from T-RFLP and DGGE of the 16S rRNA gene, showed differences in the archaeal community structure in charcoal fragments when compared to ADJ soils. The phyla Crenarchaeota and Euryarchaeota were detected in ADE and ADJ soils, and in charcoal fragments. The Thermoprotei was the only class found in this study. This was the first study to investigate archaeal community diversity present in charcoal fragments from ADE soils by using the pyrosequencing technology. However, the present results suggest further studies and the requirement of a deeper assessment, especially of isolation/cultivation of archaea, for a better understanding of the ecological and functional role of these microorganisms focusing on the biogeochemical cycles.

006B Abundance and diversity of nitrifying prokaryotes in drinking water treatment plants
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Ammonia in source water increases chlorine demand in drinking water treatment process and also increases the potential of disinfection by-products. Nitrification is a commonly used process for ammonia removal in wastewater treatment, consisting of two-steps reactions including oxidation of ammonia to nitrite and further oxidation of nitrite to nitrate. This application in drinking water treatment, however, is relatively few and information is limited on nitrifying prokaryotes in drinking water treatment processes. Furthermore, the information for ammonia-oxidizing archaea that was demonstrated to have advantage than ammonia oxidizing bacteria in low ammonia environment, such as drinking water treatment process, is also unavailable.

In order to better understand nitrification in drinking water treatment processes, this study was motivated to investigate abundance and diversity of nitrifying prokaryotes by using real-time PCR and terminal restriction fragment length polymorphism. In general, real-time PCR results showed that ammonia-oxidizing archaea and Nitrospira were dominant ammonia and nitrite oxidizers in the samples taken from the sand filtration and biological activated carbon processes, but ammonia-oxidizing archaea and ammonia-oxidizing bacteria were equally low in the samples from the plant B-1 which practiced pre-chlorination for ammonia removal. It is likely that pre-chlorination may affect the abundance of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in the filter media. The impact of temperature on ammonia-oxidizing archaea and ammonia-oxidizing bacteria abundance in the sand
filter and biological activated carbon samples was not apparent, but high temperature seemed to favor the growth of ammonia-oxidizing archaea over ammonia-oxidizing bacteria onto the sand filter and biological activated carbon media. Results of terminal restriction fragment length polymorphism analyses for ammonia-oxidizing archaea indicated that terminal restriction fragment 220 bp was the ubiquitous dominant ammonia-oxidizing archaea in almost all samples examined, while terminal restriction fragment 169 bp appeared to be prevailing occasionally.

007B  Archaeal and Bacterial Abundance Patterns in Sediments of Aarhus Bay, Denmark

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Marine sediment is one of the largest prokaryotic habitats on earth. Previous studies have shown that sediment geochemistry determines the abundance and composition of microbial communities. Yet, fundamental questions, such as whether Archaea or Bacteria are more abundant, remain contested. Here we identify abundance patterns in archaeal and bacterial distribution in relation to sediment stratigraphy and dominant redox processes across a transect of 8 stations in Aarhus Bay using quantitative PCR. Distributions are similar across all stations: archaeal abundance is low in surface sediment, then increases, reaching a peak within the upper 1m, and decreases again below. In contrast, bacterial abundance is always highest in surface sediment and then decreases with increasing sediment depth. At stations where the methanogenesis zone was reached, localized small archaeal and bacterial abundance peaks were found in the sulphate - methane transition zone. Consistent with trends in total abundances, the ratio of Archaea to Bacteria is lowest near the sediment surface, in the uppermost 10-20 cm (ratio: 0.03-0.08). This depth interval corresponds to the depth of high bioturbation, and indicates a potentially important role of infaunal invertebrates in controlling absolute and relative abundances of Bacteria and Archaea. Below this surface layer, Archaea become as abundant as Bacteria. In methanogenic deeper sediment, the ratio of Archaea to Bacteria shifts even further towards Archaea, reaching a factor of up to 5, and highlighting the importance of Archaea in methanogenic subsurface sediments.

008B  Representatives of the uncultured Euryarchaeota Clade LDS prevail among filterable Archaea in northern freshwater habitats

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As shown by number of recent studies, water sampled from various freshwater habitats contains a large number of ultra-small microbial cells, which can pass via 0.2 µm-pore-size filters. The diversity and biology of these filterable cells is poorly understood. Only a minor part of these microorganisms can be cultured in the laboratory, while vast majority of ultra-small microbial forms remain uncultured and uncharacterized. In this study, we focused on assessing phylogenetic diversity of filterable archaea, which occur in water of various boreal freshwater habitats.

Water samples from two boreal acidic lakes, dystrophic Lake Dubrovskoe and oligomesotrophic Lake Motykino, ombrotrophic peat bog Obukhovskoe, and artificial water reservoir Rybinskoe (Yaroslav and Vologda regions, European North Russia) were used for the molecular diversity analysis. Water samples were passed through 0.2-µm-pore-size membrane filters. Cells that were not captured by these filters were collected by further filtration via 0.1-µm-pore-size membranes. The latter were taken for DNA extraction using «FastDNA SPIN kit for soil» (Biol 101, USA). PCR amplification of 16S rRNA genes was performed using Archaea-specific primers 109f and 915r. PCR products were cloned, sorted out by restriction fragment length polymorphisms analysis and sequenced.

In total, 301 archaeal 16S rRNA gene sequences were retrieved from the filterable cell fraction. Only four of these sequences affiliated with the Thaumarchaeota, while remaining 297 sequences belonged to the phylum Euryarchaeota. Of these, 134 16S rRNA gene sequences displayed high (97-99%) similarity to those in taxonomically described methanogenic archaea of the orders Methanobacteriales, Methanomicrobiales, and Methanosarcinales. This appears logical since some representatives of these archaeal groups, including members of the genera Methanobacterium and Methanoregula, have very thin (0.1-0.3 µm in diameter) cells. Another half of cloned 16S rRNA gene sequences from filterable archaea affiliated with two uncultured mesophilic Euryarchaeota clades, Lake Dagow
Sediment (LDS) and Rice Cluster V (RC-V). The most abundant group of sequences (154 clones) belonged to the LDS clade. Notably, these LDS-related 16S rRNA gene sequences were retrieved from all our sampling sites, i.e. boreal lakes, peat bog, and artificial water reservoir, and displayed high level of genetic diversity. In the phylogenetic tree, these sequences formed four separate lineages, which displayed 10-18% of sequence difference. Each of these four lineages included representative sequences from each of the habitats examined in our study as well as a number of 16S rRNA gene sequences that have earlier been recovered from various freshwater, mostly oxic and cold environments. Our data suggest that the enigmatic LDS clade is represented by ultramicro-sized archaea, which tend to inhabit freshwater northern ecosystems.

009B  Enrichment and characterization of three freshwater ammonia-oxidizing archaea
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Ammonia oxidation is the first and rate limiting step in nitrification, the process of converting nitrogen from its most reduced form (ammonium) to its most oxidized form (nitrate). Aerobic ammonia oxidation was previously thought to be carried out only by members of the Beta- and Gamma-Proteobacteria. Recently, ammonia oxidation was discovered among Archaea as well. We have enriched three ammonia-oxidizing archaea from two freshwater lake sediments in Ohio, USA. The three enriched ammonia-oxidizing archaea belong the Thaumarchaeal group I.1a. Enrichment AC-2 is closely related to Candidatus Nitrosoarchaeum koreensis (99.6% nucleotide identity to 16S rDNA), while enrichments AC-5 and DW-1 represent a new genus. We compared the growth of these archaeal enrichments to that of the ammonia-oxidizing bacterial enrichment culture G5-7, which contains Nitrosomonas sp. Ls79A3, which was enriched from a freshwater lake in the Netherlands. We experimentally cultured each enrichment in differing concentrations of ammonium, oxygen, as well as a range of pH and light treatments. Nitrite/nitrate production was determined colorimetrically and used to calculate the growth rates. Our data reflect that all of the ammonia-oxidizing archaeal enrichments grow faster in lower ammonium concentrations (15 µM to 0.5 mM), and are significantly hindered by ammonium concentrations higher than 2 mM. The AOB enrichment G5-7 shows increasing growth rates with increasing ammonium concentration, with maximal growth rates at 1 mM ammonium. The growth of G5-7 shows a clear optimum at pH 7.5, while the archaeal cultures show differential growth patterns over the investigated range of pH (6 to 9). The ammonia-oxidizing archaeal enrichments AC-5 and DW-1 were not inhibited by low oxygen concentrations (0.5-2%). Enrichment AC-2 did not grow at oxygen concentrations lower than 1%, however growth from 1% to atmospheric conditions occurred at a constant rate. In contrast, the ammonia-oxidizing bacteria G5-7 had significantly reduced growth under low oxygen concentrations. In the presence of white light at 30 µmol photons/m²/s, the ammonia-oxidizing bacterial enrichment G5-7 grew at the same rate as in the dark, while growth all three of the ammonia-oxidizing archaeal enrichments was inhibited. Both the ammonia-oxidizing bacteria and the ammonia-oxidizing archaea were not inhibited by red light, and were incapable of growth in the presence of blue light. Overall, our results show that the growth of freshwater ammonia-oxidizing archaea favors conditions of low substrate supply, which may allow for niche separation between ammonia-oxidizing archaea and ammonia-oxidizing bacteria in freshwater environments. In the future we plan to conduct competition experiments between the ammonia-oxidizing bacterial and ammonia-oxidizing archaeal enrichment cultures in laboratory and in situ conditions.

010B  Methanogenic archaea in a Sub-Arctic acidic palsa peatland
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Peatlands store huge amounts of carbon and cover around 3% of the terrestrial surface with a main distribution in northern high latitudes. Northern peatlands of permafrost landscapes such as palsa peatlands are currently subjected to geomorphological and biogeochemical changes due to climate change leading to release of greenhouse gases including methane (CH₄). The CH₄ >emission is a balance between production and consumption. In order to understand and predict CH₄ emissions from permafrost-affected peatlands, we have to understand how the CH₄ cycling communities respond to permafrost degradation. Here, we focused on the CH₄ producers, the methanogenic archaea, in a degrading palsa peatland.
Two contrasting peatland sites (NE1, NE2) located near Neiden (Finnmark, Norway) were investigated vertically for their potential methane production, environmental parameters (e.g., pH, C/N) and methanogenic community structure based on 16S rRNA and the functional mcrA gene. Site NE1 was a thermokarst pond next to a degrading palsa whereas site NE2 was a stabilized collapsed site. NE1 was characterized by pH values of around pH 4.2 and NE2 of around pH 4.8. Both sites can be described as highly oligotrophic with C/N values between 37 and 97. The vegetation was dominated by Sphagnum with Eriophorum mainly growing in the thermokarst pond and Carex at the stabilized collapsed site. The potential methane production was low for all depths without substrate. Additional substrate (H₂/CO₂, acetate) stimulated the CH₄ production in all samples resulting in higher values in the thermokarst compared to the stabilized site, especially after the addition of acetate.

We detected methanogenic archaea related to the orders Methanobacteriales, Methanosarcinales, Methanomicrobiales and Methanocellales. The overall diversity of methanogenic archaea was low with only 5 OTUs based on deduced mcrA amino acid sequences. Most of the sequences were related to environmental sequences that could be detected in other acidic peatlands. The thermokarst pond in front of the degrading palsa was characterized by a methanogenic community that changed from mainly hydrogenotrophic in the upper layer to a mainly acetoclastic community in the lower depths. For the collapsed stabilized site the methanogenic community was mainly hydrogenotrophic at all depths.

Our results show that air temperature induced changes in geomorphology, hydrology, vegetation, and nutrients will probably alter the community structure of methanogenic archaea in palsa ecosystems. Also, our study indicates that the formation of thermokarst initially leads to an increased potential to produce methane in particular through acetoclastic methanogens which again slows down and shifts towards a more hydrogenotrophic community after the stabilization of the disturbed palsa sites.

011B Towards understanding geomicrobiology of an extreme environment: characterization of a novel Acidianus species involved in iron and sulphur cycles isolated from Copahue-Caviahue acidic geothermal area

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Extreme environments are being studied all over the world by many different approaches, particularly the -omics techniques. However, in order to understand how microorganisms relate and impact on environment, it is useful to have them isolated and deeply characterized. Copahue-Caviahue is a geothermal region under the influence of active Copahue volcano. In this area there are many hot springs with different physicochemical conditions that also have different prokaryotic biodiversity. This natural extreme environment is an ideal place to look for new, yet undiscovered species that might help to clarify the role of microorganisms in different element natural cycles. Besides, novel species isolated from acidic high temperature environments might have many potential biotechnological uses.

In this work we present a novel crenarchaea isolated from three separated acidic geothermal hot springs from Copahue-Caviahue region. ALE1 isolate is a thermoacidophilic Acidianus able to develop at a wide range of pH and temperatures, from 1.0 to 5.0 and 55° to 80° C respectively. Growth rate and culture cell density are affected by these parameters, and optimal conditions are 75°C and pH 2.5 – 3.0. One of the most surprising features of this isolate is its great ability to develop using different energy and carbon sources; it is able to grow aerobically on sulphur, tetrathionate, iron (II) and sucrose. As all members of Acidianus genus ALE1 can also develop under anaerobic conditions, but this isolate can use a wider range of substrates than the other Acidianus so far reported; it grew autotrophically using ferric iron or sulphur as electron acceptors and sulphur or hydrogen as electron donors. AFM, TEM and SEM microscopy showed ALE1 cells irregular coccus form surrounded by a regularly arrayed glycoprotein layer (S-layer). Another peculiar characteristic of this novel archaea is that it was isolated from three separated and independent hot springs (and detected by non culture methods in a fourth) but the comparison of its 16S rRNA gene sequence in NCBI data base did not show any close relatives, cultured or uncultured, found elsewhere. It appears that ALE1 is an autochthonous microorganism from Copahue-Caviahue region. Its closest related species are strains of Acidianus hospitalis (91 % of sequence similarity), Acidianus infernus (90 %), Acidianus ambivalens (90 %) and Acidianus manzanensis (90 %). As regards the isolate biotechnological uses, its ability to
oxidise sulphur compounds and ferrous iron at high temperature makes it potentially very useful tool for bioleaching and biooxidation, especially of refractory ores like molybdenite and chalcopyrite which are scarcely dissolved by mesophilic microorganisms.

ALE1 isolate growth flexibility towards pH and temperature and its ability to use a variety of oxidation state forms of sulphur and iron, auto and heterotrophically, in aerobic and anaerobic metabolisms points to its unique role in an environment that, due to its particular physicochemical and geological conditions presents niches with all these characteristics. Even more, these different microenvironments could have been originated by metabolic activities of microorganisms like ALE1.

012B  Niche specialization of terrestrial thaumarchaea
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Soil pH is a major determinant of microbial ecosystem processes and potentially a major driver of evolution, adaptation and diversity. In this study we focused on the thaumarchaea, an abundant archaeal evolutionary lineage, which contributes significantly to nitrification, a critical step in terrestrial nitrogen cycling. While the low number of cultivated representatives has limited investigation of these organisms, high-throughput sequencing of amplified functional genes makes it possible to understand their diversity, distribution and adaptation to the environment. To determine whether pH drives evolutionary adaptation and community structure of soil archaeal ammonia oxidizers, sequences of amoA, a key functional gene of ammonia oxidation, were examined in soils at global, regional and local scales. A global meta-study dataset and regional and local datasets obtained by high-throughput sequencing of amplicons derived from 47 UK soils clustered into 19 well-supported phylogenetic lineages (defined at 90% similarity level) that dominated specific soil pH ranges classified as acidic (pH distribution of these amoA gene-defined lineages. Furthermore, archaeal amoA gene abundance and diversity increased with soil pH, which was the only physicochemical characteristic measured that significantly influenced community structure. These results suggest evolution based on specific adaptations to soil pH and niche specialization resulting in a global distribution of archaeal lineages that have important consequences for soil ecosystem function and nitrogen cycling.

In order to determine whether different environmental factors influence thaumarchaeal adaptation at different taxonomic levels, this dataset was being analysed at six other phylogenetic scales. Additional environmental factors explain the distribution of the thaumarchaeal community at different taxonomic levels, suggesting a ranking of these factors in term of their importance for microbial adaptation.

013B  Archaea diversity and their roles on nitrogen cycling at extreme lake
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The phylogeny of the latest recognized domain, Archaea, is still complicated and it is largely based on environmental sequences. Recent data suggest that the Archaea provide the major routes for ammonia oxidation in the environment. Here, we focus on archaeal diversity and their roles on the nitrogen cycling at extreme lakes.

Sampling areas are Salda Lake is highly alkaline lake (pH=9.8) and Aci Lake is hypersaline lake (%0 35.0) from Turkey. Archael diversity is determined by pyrosequencing analyses. Gene of nitrogen cycling are amoA archaea is screening by PCR.

Consequently, the constructed archaeal libraries are characterized by high proportion of OTUs representing uncultivated archaeal phylogroups, the abundance of novel phylotype sequences, the presence of high proportions of Crenarchaeota phylotypes unrelated to cultivated organisms. amoA archaea genes submitted at NCBI database.
Recent studies from marine and terrestrial environments suggest that ammonia-oxidizing archaea are better adapted to conditions of low ammonia availability than ammonia-oxidizing bacteria. However, only little is known about the respective role of archaeal versus bacterial ammonia oxidizers in freshwater environments. We carried out comparative investigations of the abundance, community composition, and activity of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in sediments of a eutrophic and a neighbouring oligotrophic lake located in Northwest Germany, addressing two hypotheses: (i) Ratios of archaeal and bacterial ammonia oxidizers shift in favour of ammonia-oxidizing bacteria with increasing ammonium availability, and (ii) ammonia-oxidizing archaea play a major role in ammonia oxidation in the oligotrophic lake while ammonia-oxidizing bacteria dominate this process in the eutrophic lake. Abundance, transcriptional activity, and community composition of archaeal and bacterial ammonia oxidizers were analyzed targeting the *amoA* gene encoding ammonia-monooxygenase as a functional marker. Archaeal *amoA*/bacterial *amoA* gene ratios ranged from 0.001 to 1 in the sediment of the eutrophic lake and from 1 to 1000 in the oligotrophic lake. Here, ammonia-oxidizing archaea were especially abundant in rhizosphere sediment where they constituted up to 50% of the total archaeal population. Sediment samples taken directly from the field site or from short-term incubation experiments showed higher transcript numbers of archaeal *amoA* or bacterial *amoA* in the eutrophic and eutrophic sediments, respectively. NH$_4^+$ concentrations in the sediment pore water were negatively correlated with archaeal *amoA*/bacterial *amoA* gene ratios and positively correlated with bacterial *amoA* gene copy numbers across sites. Up to 30 times higher potential nitrification rates in the eutrophic compared to the oligotrophic sediments coincided with higher abundances of bacterial *amoA* genes. Preincubation of samples with antibiotics targeting ammonia-oxidizing bacteria resulted in a reduction of nitrification activity by up to 70% in the eutrophic sediments while almost no reduction was observed in the oligotrophic sediments. Both the molecular and the activity data suggest an increasing contribution of ammonia-oxidizing archaea to nitrification processes along a gradient from eutrophic to oligotrophic conditions.

The discovery that Archaea are significant and sometimes dominant members of marine microbial populations, along with the finding of the genetic coding for the enzymes of nitrification, has led to the assumption that Archaea are the main organisms responsible for ammonium oxidation in the ocean. Consequently there has been a renewed interest in rate measurements of natural populations. However, to correct rate measurements to the in-situ rate it is necessary to understand concentration and temperature dependencies. To investigate these functions we determined the ammonium oxidation rate dependence on total ammonium concentration (NH$_3$+NH$_4^+$) and its temperature dependence of naturally occurring marine communities. Rates were measured on whole water samples using 15N-ammonium tracer addition/incubation techniques. The vertical distributions of ammonium oxidizing Archaea (AOA) and bacteria (AOB) were determined by Q-PCR (AmoA gene). Measurements were made in Puget Sound, Washington, USA at a 130 m deep location during June and July 2011. AMO gene copy numbers suggested that the ammonium oxidizing communities were dominated by Archaea, $10^5$ copies/ml, rather than Bacteria, $10^3$ copies/ml, except at the surface where gene copy numbers were about equal. In all cases the rate dependence on ammonium concentration followed Michaelis-Menten-type kinetics. Half saturation constants, Km, showed a high affinity for ammonium with values of about 125 nM. 15N-ammonium oxidation rates were constant with time except in those cases where substrate limitation developed near the end of an incubation. In situ ammonium oxidation rates were near zero at the surface and increased with depth up to values near 300 nM/d. The temperature dependence of ammonium oxidation rate shows a maximum at 18°C and had a Q10 of 2.0. Below the euphotic zone rates were generally consistent with ammonium production rates estimated from oxygen consumption and Redfield stoichiometry. Our measured Km values were similar to previously published Km values for the Archaeal nitrifier Nitrosopumilus of about 130 nM. Thus, our Km values for the natural populations of Puget Sound coupled with the AOA and AOB gene copy numbers suggest AOA are responsible for ammonium oxidation in this environment.
286B  Niche partitioning of marine group i crenarchaeota in the euphotic and upper mesopelagic zones of the east China sea

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Marine Crenarchaeota Group I (MGI) is a ubiquitous and numerically predominant microbial population in marine environments. Understanding of the spatial dynamics of MGI and its controlling mechanisms is essential for understanding of the role of MGI in energy and element cycling in the ocean. In the present study, we investigated the diversity and abundance of MGI in the East China Sea (ECS) by analysis of crenarchaeal 16S rRNA genes, the ammonia monoxygenase gene amoA and the biotin carboxylase gene accA. Quantitative PCR analyses revealed that these genes were higher in abundance in the mesopelagic than in the euphotic zone. In addition, the crenarchaeal amoA gene was positively correlated with the copy number of the MGI 16S rRNA gene, suggesting that most of the MGI in the ECS are nitrifiers. Furthermore, the ratios of crenarchaeal accA to amoA or to MGI 16S rRNA gene abundance increased from the euphotic to mesopelagic zones, suggesting that the role of MGI in carbon cycling may change from the epi- to the meso zones. Denaturing gradient gel electrophoretic profiling of the 16S rRNA genes revealed depth partitioning in MGI community structures. Clone libraries of crenarchaeal amoA and accA genes showed both ‘shallow’ and ‘deep’ groups and their relative abundance varied in the water column. Ecotype simulation analysis revealed that MGI in the upper ocean could diverge into special ecotypes associated with depth to adapt to the light gradient across the water column. Overall, our results showed niche partitioning of the MGI population and suggested a shift in their ecological function between the euphotic and mesopelagic zones of the ECS.

016B  Archaeal nitrification in acidic forest soils receiving high atmospheric N deposition

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It has been believed that autotrophic NH3 oxidizing bacteria (AOB) and heterotrophic microbes are responsible for nitrification in acidic forest soils. However, since the recent discovery of NH3 oxidizing archaea (AOA), AOA could now be the probable candidate mainly responsible for the reaction. Worldwide N deposition due to anthropogenic activities is predicted to increase, with the largest absolute increases occurring over East and South Asia. Chronic N deposition drastically modifies N cycling in forest ecosystems and causes N-saturation state with increasing net soil nitrification and subsequent N losses via NO3-leaching, and also accelerating soil acidification. The identification and physiological characterization of the microbial group mainly responsible for the nitrification in such acidified forest soils allows a better understanding of the mechanisms underlying these ecological phenomena.

The study sites were set in N-saturated and non-saturated broadleaf forests and non-saturated pine forests receiving high atmospheric and manipulated N depositions in southern China. The soils were acidic (pH 3.6-4.2). Seriously high concentration of NO3 in the soil (14.4 mg-N kg-soil−1) and the stream (4.3 mg-N l−1) in the N-saturated broadleaf forest has been observed. To investigate the contribution of autotrophic and heterotrophic microbes to nitrification, gross nitrification rates in the soils were assessed by using 15NO3 and the inhibitor for autotrophic nitrification, C2H2. Abundances of gene transcript of ammonia monoxygenase (amoA) of AOA and AOB were quantified by using mRNA extracted from the soils. To track the source of NO3 in the stream, delta 15N and 18O of NO3 in the stream water, precipitation and soil extract were analyzed.

C2H2-inhibition techniques showed that gross rates of heterotrophic nitrification were quite small compared with autotrophic nitrification in all the soils, suggesting the dominant contribution of autotrophic nitrifier. Abundances of amoA gene transcript of AOA in the soils were extremely high (1.6×109 - 2.6×109 g-soil−1) while those of AOB were not detected. Furthermore, the abundances were positively correlated with the autotrophic nitrification rates among all examined soils. This strongly suggested that AOA rather than AOB be mainly responsible for the nitrification in the soils. Comparing the results in this study with those in previous studies allowed the implication that ammonia oxidation
activity per cell of AOA was approximately $10^3$ lower than that of AOB. The largest rate and highest abundance was observed in the soils of the N-saturated broadleaf forest. Isotope analysis of NO$_3^-$ showed that NO$_3^-$ in the stream was derived mainly from NO$_3^-$ produced via nitrification in the soil and leached, but not from precipitated NO$_3^-$. These results insisted that high concentration of NO$_3^-$ observed in the soil and the stream was mainly due to high abundance of active AOA despite its low activity per cell. Our results suggested that archaea could play leading roles in N transformations and loss in acidic forest soils receiving high atmospheric N deposition.

017B  Enrichment and characterization of autotrophic ammonia-oxidizing thaumarchaea from an agricultural soil

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Nitrification of excess ammonia in soil causes eutrophication of water resources and emission of atmospheric N$_2$O gas. The first step of nitrification, ammonia oxidation, is mediated by Archaea as well as Bacteria. The physiological reactions mediated by ammonia-oxidizing archaea (AOA) and their contribution to soil nitrification are still unclear. We obtained two highly enriched ammonia-oxidizing cultures (MY1 and JG1, respectively) containing group I.1a and I.1b thaumarchaea from an agricultural soil dominated by a single archaeal population [ca. 90% of total cells, as determined microscopically (by FISH) and by quantitative PCR of its 16S rRNA gene]. No bacterial amoA genes could be detected by PCR. MY1 culture contained an archaeon, "Nitrosoarchaeum koreensis MY1", fell phylogenetically within crenarchaeal group I.1a; sequence comparisons to "Nitrosopumilus maritimus" revealed 96.9% 16S rRNA and 89.2% amoA gene similarities, respectively. JG1 culture contained "Nitrososphaera sp. strain JG1" fell within thauamarchaeotal group I.1b and was related to the moderately thermophilic archaean, "Nitrospahera gargensis", and the mesophilic archaean, "Nitrospahera viennensis" with 97.0% and 99.1% 16S rRNA gene sequence similarity, respectively. A $^{13}$C-bicarbonate-assimilation assay showed chemolithotrophic growth of AOA with stoichiometric incorporation of $^{13}$C into Archaea-specific glycerol dialkyl glycerol tetraethers. Growth assays showed cultivated soil AOA were neutrophilic, non-halophilic, and mesophilic. The optimum temperature of strain JG1 (35-40°C) was >10°C higher than that of ammonia-oxidizing bacteria (AOB). Soil AOA produced a significant amount of greenhouse gas, N$_2$O, comparable to those of soil AOB.

018B  Methanogenesis in deep warm groundwater

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The relatively low stable isotope signature of delta-$^{13}$CH$_4$ (-54.0%) showed the bubble of methane obtained from the groundwater at 140 m and 500 m deep in sedimentary geological setting of Hokkaido, North Island of Japan was partially made of microbial activity. Incubation experiments with the 500 m deep water indicated the highest activity of methanogenesis was carried out at 30° and 40°/45°C under the anoxic pseudo in situ condition in a month, but any methanogenesis was detected at neither 20°C nor 55°C. Assuming increasing in temperature with 4°C/100m and 15°C for the surface groundwater temperature increases to 35°C in 500 m. Actually the measured temperature of groundwater in 500 m was 31.5°C there. On the other side, the water obtained from 140 m deep did not show any methanogenic activity in the incubation with in situ temperature of 15°C and 30°C for more than three months, while the presence of the candidate archaea was shown for the both water by gene sequence of small subunit rRNA.

When the water of 30 ml carefully taken from 500m deep of a borehole using a double packer sampler was incubated in each glass bottle which had been kept vacuum condition and filled the headspace with nitrogen gas with addingmethanol under different temperature, CH$_4$ was detected in 10 days at 30° and 40°C and production of methane saturated from 20 to 30 days. This trend had been observed in different sampling of the same depth on October of 19, 2007. Produced methane of 4 mM indicated the added methanol of 10 mM was completely consumed by converting to methane.
Since exhaustion of added methanol another methanogenic activity appeared within two months in a bottle incubated with methanol in water and CO\textsubscript{2}/H\textsubscript{2} in headspace. This suggested a sort of different utilization of substrates occurred in archaeal community of 500 m deep. Methanol was utilized first as an electron donor, which was followed by hydrogen. Gene sequence showed candidates being able to utilize both substrates existed in the examined groundwater. Other substrates as acetate and formate were shown to be secondary electron donors utilized slightly after two months.

**019B** Prevalence and diversity of ammonia-oxidising archaea and bacteria during composting of municipal waste  
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Many European states have implemented new regulations for municipal waste, requiring waste accepted for landfill to first undergo aerobic composting (biostabilisation) in order to reduce its biodegradable component. Application of these guidelines has created a critical need in the waste-management industry to look into possible methods of accelerating biostabilisation. Although many strategies for accelerating degradation of organic waste are known, limited research has been conducted on municipal waste, and little is known about the microbial dynamics of the process.

In the past few years, microbial ecologists have become aware that ammonia-oxidation, a key step in the nitrogen cycle, is carried out not only by autotrophic bacteria, but also by members of the Crenarchaeota. As this phenomenon was studied, it was discovered that archaea are often the predominant players in ammonia-oxidation in soil environments. However, very little is known about the contribution of archaea to ammonia oxidation in composting systems. Although it is logical to presume they play an important role in composting as they do in soils, previous studies have been unable to detect ammonia-oxidising archaea in composts. As the nitrogen status of compost is crucial to its use as a fertiliser, it is important to ascertain whether archaea play a role in the key nitrifying step of ammonia-oxidation.

We present the first study to investigate the prevalence and diversity of bacterial and archaeal amoA genes in municipal waste compost. The effect of various commercially-feasible manipulations (including additions of lime and green waste) on the rate of biostabilisation of the fine (<20 mm) fraction of mixed municipal waste was investigated. Samples were taken at five stages during the composting process, on days 0, 6 (thermophilic phase), 28 (end of thermophilic phase), 41 (cooling phase), and 57 (maturity). The physical and chemical attributes of the compost samples were measured, and their bacterial and fungal communities profiled using traditional culture-based methods. The prevalence and diversity of archaeal and bacterial amoA genes were assessed by real-time PCR and denaturing gradient gel electrophoresis, respectively.

It was found that the composting amendments affected the time it took the compost to mature, with more rapid decomposition occurring in composts treated with lime and green waste. The diversity and abundance of both archaeal and bacterial ammonia-oxidisers were affected by amendment, and by composting stage. For most stages of composting, the archaeal amoA community was found to be more diverse than the bacterial amoA community. Significant differences were also noted between copy numbers of archaeal and bacterial amoA genes. The results suggest that archaeal ammonia-oxidisers play a key role in nitrogen cycling in municipal waste compost, and that their diversity and abundance can be influenced by accelerating amendments.

**020B** Archaea of the Miscellaneous Crenarchaeotal Group (MCG) are abundant, diverse, and widespread in marine sediments  
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Members of the highly diverse Miscellaneous Crenarchaeotal Group (MCG) are globally distributed in various marine and continental habitats. In this study we applied a polyphasic approach (rRNA slot
blot hybridization, quantitative PCR, and CARD-FISH) using newly developed probes and primers for the in situ detection and quantification of MCG crenarchaeota in diverse types of marine sediments and microbial mats. In general, abundance of MCG (small cocci, 0.4 to 0.5 µm in diameter) relative to other prokaryotes was highest with 12-100% in anoxic, low-energy environments characterized by deeper sulfate depletion and lower microbial respiration rates. When studied in high depth resolution in the White Oak River estuary and Hydrate Ridge methane seeps, MCG abundance relative to total archaea and MCG phylogenetic composition did not correlate with changes in sulfate reduction or methane oxidation with depth. In addition, MCG abundance did not vary significantly (p > 0.1) between seep (with high rates of methanotrophy) and non-seep sites (with low rates of methanotrophy). This suggests that MCG are likely not methanotrophs. MCG crenarchaeota were highly diverse and contain 17 subgroups, with a range of intragroup similarity of 82 to 94%. This high diversity and widespread distribution in subsurface sediments indicates that this group is globally important in sedimentary processes.

021B  Effect of carbon source on microbial community structure in a high cell density anaerobic reactor
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Study of factors influencing microbial community structure, which is highly sensitive but poorly understood; contribute largely to increasing popularity of anaerobic digestion as an emerging bio-energy technology in wider field applications. The fact underlying stable performance of reactor is stable and metabolically active microbial community, besides sophisticated reactor engineering. This study investigated the correlation of community structure with reactor's performance and operating conditions. Self-immobilized granules were developed on simulated wastewater based on different carbon sources (glucose, molasses and milk-based) and maintained in fluidized condition in reactors operated at mesophilic condition. Despite the fact that difference in wastewater composition shapes up community structure, methanogenic profile (using PCR-denaturing gradient gel electrophoresis approach (DGGE) with 16S rRNA gene amplicons) of samples withdrawn from different reactors did not indicate drastic qualitative differences in the structure of methanogenic community. Differences were observed only with respect to relative intensities of the bands. Metanosaetaceae sp. was the most dominant among total 10 identified operational taxonomical units (OTU). However, DGGE profiles of total bacterial community revealed differences between samples from various carbon sources tested. Clostridium sp. was the most dominant one among the identified OTUs. An insight into the acetogenic population, by DGGE profiling, showed commonly found acetogens in anaerobic digestion. Study of spatial distribution of communities within the granule is being attempted by fluorescence in-situ hybridisation of the granules with universal archaea and bacterial oligonucleotide probes targeting conserved domain of 16S rRNA gene. Further, to study the effect of shock load on microbial community profiles, hydraulic retention time (HRT) was decreased for each reactor. The community profiles generated by DGGE exhibited no change with increasing shock load during the stable performance of any of the reactors. But shock load beyond reactors' tolerance level resulted in significant changes in the methanogenic community profile during both transient and steady states. The changes in the profile can be directly linked to predict system disturbances and help prevent the complete deterioration of the system. In spite of the same community structure in all reactors, the milk and molasses based reactors showed better tolerance level towards the shock loads compared to glucose based one as metabolism of glucose is faster. The study signifies the fact that microbial community profiles, especially fingerprints targeting methanogens, can be good markers to reflect the system status and can be used efficiently as a preventive tool to avoid complete failure of the system. In the near future integration of molecular biology approaches and reactor engineering holds lot of promise in solving the existing challenges in anaerobic digestion.

022B  The existence of ammonia-oxidizing archaea in wastewater treatment plants
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Until recently, ammonia oxidation was considered to be performed largely by autotrophic ammonia oxidizing bacteria (AOB), Anammox bacteria. However it has been shown that ammonia oxidation is also performed by ammonia oxidizing archaea (AOA) and not restricted to the bacteria domain. In addition, culture dependent surveys and quantitative real time PCR studies indicate that Archaeal amoA gene is much more abundant and active than their bacterial counterparts in various
environmental samples. AOA might also play an important role in biological nitrogen removal reactors of waste water treatment plants. Although the wide distribution of AOA in the environment and their abundance over AOB is currently well established; only a few studies focus on the presence, operating conditions and activity of AOA in engineered systems. The limited research on AOA activity in wastewater treatment plants points out the importance of operating conditions of the reactors.

Accordingly, in this study, the presence of AOA in engineered systems were investigated using two primer sets specific for archaeal amoA gene sequences via conventional and quantitative real time PCR. For this purpose activated sludge samples from domestic and industrial wastewater treatment plants, and as a positive control various environmental samples were screened. In addition, three different strains of AOA enrichment cultures were incubated and analyzed to determine optimal growth conditions for AOA in different ammonium concentrations.

Activated sludge samples were collected from 16 different domestic and industrial wastewater treatment plants where nitrification was active, including petroleum and oil refineries; food, alcohol and chemical industries and landfill leachate treatment plants.

Out of 16 wastewater treatment plants; at only one domestic wastewater treatment plant AOA occurrence has been found. Unlike environmental samples, there is no molecular evidence on AOA abundance in engineered systems has been observed via conventional PCR. Thus both the detection sensitivity of the conventional PCR and the ammonia level in engineered systems has been searched to be able to answer why such AOA diversity not found in wastewater systems?

As a result of enrichment culture studies; AOA grows optimum at ~1mM ammonia concentration. Cell growth is greatly retarded at ~10mM and inhibited at ~20 mM ammonia concentrations. As in concordance with our full scale sample results, found only in domestic wastewater treatment plant where the ammonium levels are below 1mM.

On the other hand, quantitative real time PCR methodology has been applied to be able to check detection limit of conventional PCR over AOA occurrence. Surprisingly in almost all of the samples, AOA occurrence has been showed within the 10³ - 10⁸ copy numbers.

It has been confirmed that conventional parameters such as NH₃ concentration might be a good tool to predict AOA occurrence in engineered systems. As a result, AOA has been found in industrial engineered systems, whereas it may not be considered as a potential nitrifier in wastewater treatment plants.

**023B Acidophilic ammonia oxidising archaea: explaining high rates of nitrification in low pH soils?**
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Ammonia oxidation is the first and rate-limiting step in nitrification and constitutes an essential part of the global nitrogen cycle. Approximately 30% of the world's soils are considered acidic (pH13-CO₂ after stable isotope probing. Unlike previously described ammonia-oxidisers, the growth rate of this organism is negatively correlated with ammonium concentration and growth occurs at extremely low ammonia concentration. To gain further understanding into the mechanisms enabling growth and ammonia oxidation in acidic conditions, DNA from batch cultures was subjected to high-throughput sequencing. A draft genome of Nitrosotalea devanaterra was assembled using MIRA with a preliminary annotation generated using a series of automatic pipelines. The genome of Nitrosotalea devanaterra contains numerous genes absent from other neutrophilic ammonia-oxidising archaea such as Nitrosopumilus maritimus and Nitrosoarchaeum limnia, which may play a role in adaptation to growth at low pH. Genes encoding ATP-driven potassium pumps, as well as several other cation transporters are present and may be involved in producing a reversed membrane potential that prevents acidification of the cytoplasm. While amt-type ammonium transporters found in other ammonia-oxidising archaea are encoded by the genome of Nitrosotalea devanaterra, there are also unique transporters potentially involved in nutrient uptake. The cultivation of Nitrosotalea devanaterra provides a previously unsuspected explanation for nitrification in acidic soils and its genome suggests multiple mechanisms enabling growth at low pH.
Opening the archaeal box in an oligotrophic freshwater environment
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Owing to the wide use of both culture-dependent and -independent techniques, knowledge on the biology of Archaea is rapidly increasing widening capabilities to detect and culture them. An important role for some archaeal groups (for example methane (CH4)-related and ammonia-oxidizing archaea) has been envisaged in global biogeochemical cycles. However, many challenges still remain. Importantly, archaeal taxonomic assignations are limited due to both the lack of cultured representatives for all archaeal groups and a dearth of full-length 16S rRNA gene sequences. The objective of this research is to expand our knowledge on archaeal diversity and ecology in freshwater oligotrophic environments using Lake Kivu as a natural laboratory.

Lake Kivu (East Africa) is a deep (maximum depth 489 m), oligotrophic, and high altitude (1,463 m) lake of tectonic origin. The water column is split into two layers—aoxic mixolimnion (the oxycline situated around 20 to 60-m depth depending on the season) and an anoxic monimolimnion rich in dissolved salts, carbon dioxide and CH4 due to permanent stratification. Consequently, these water layers exhibit contrasting physico-chemical properties and they thus harbour different microbial populations.

In a previous snapshot study (2007), Archaea accounted for less than 5.0% of total prokaryotes (DAPI counts) and DGGE fingerprints revealed the presence of ammonia-oxidizers in oxic waters, whereas methanogenic lineages were most prevalent in anoxic waters. In order to gain a deeper insight into this archaeal community, water samples from different depths were collected three times (October 2010, June 2011, and January 2012) at three different pelagic sites and analysed by 454 FLX pyrosequencing (~250 bp reads) and quantitative PCR targeting 16S rRNA and distinct functional genes. A wide array of environmental metadata and process rate measurements was collected in parallel. Preliminary results revealed on average ca. 160 different operational taxonomic units (OTUs, 0.03 cutoff) in samples obtained in the main lake, whereas 22 OTUs were obtained from a separated and isolated basin. In all samples analysed to date, nearly half of retrieved OTUs were singletons suggesting that the archaeal assemblage in Lake Kivu was dominated by a few abundant OTUs. The archaeal richness was higher in the oxic-anoxic transition and in the fully anoxic water layer than in the oxic water compartment. In general, the permanent stratification of the water column has driven a niche differentiation of archaeal communities with ammonia-oxidizing Thaumarchaeota dominating the oxic water layer, putative heterotrophic phylotypes related to the Miscellaneous Crenarchaeotal Group present in the oxic-anoxic transition and Euryarchaeota methanogenic lineages dominating the anoxic layer. Quantitative analyses revealed similar vertical profiles between marine Thaumarchaeota 16S rRNA gene and archaeal amoA gene copy numbers, suggesting that the restriction of Thaumarchaeota to the oxic layer is related to their reliance on aerobic ammonium oxidation to support growth. Overall, our results on the distribution of archaeal phylotypes in Lake Kivu are consistent with observations from marine environments. Anoxic waters support greater archaeal diversity but favour euryarchaeotic species related to CH4 cycling. In contrast, oxic waters exhibit restricted diversity, supporting largely aerobic Thaumarchaeota sustained by oxidizing ammonium.

Emergent macrophytes alter the composition of the archaeal community in freshwater and saline wetlands
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Recent advances in microbial ecology have unequivocally shown a comparable contribution of Archaea and Bacteria to Carbon and Nitrogen cycles in a variety of environments, and coastal wetlands are not an exception. However, the role of plant roots on the selection of archaea and
bacteria has not been totally resolved. A paradigmatic example can be found in emergent macrophytes as potential selectors for ammonia oxidizers on root surfaces. Depending on plant type, environmental pH, nutrient and oxygen availability at the root surface and plant growth stage, different groups of bacterial and archaeal ammonia oxidizers are enriched and potentially selected. The aim of the present work was to investigate the effect of two emergent macrophytes, *Phragmites australis* and *Ruppia* sp., on the selection of distinct members of the archaeal community of two wetland systems of contrasting salinity (fresh- to saline).

One oligohaline and one euhaline lagoon from two preserved wetland areas in Spain ("Aiguamolls de l'Empordà i el Baix Ter" and "Parque Nacional de Doñana") were selected to study the archaeal community composition in both the rhizosphere and the bulk sediment. Fourteen 16S rRNA gene clone libraries were constructed from the bulk sediment and roots of the predominant macrophytes. A total of 625 non-chimeric clone sequences were analysed thus resulting in 203 OTUs (97% cutoff). In order to compare the plant and the salinity effect, samples were grouped in 4 groups: high salinity sediment, *Ruppia*-rhizosphere, low salinity sediment, and *Phragmites*-rhizosphere. All sample groups showed a high site-specificity with many exclusive OTUs and low shared diversity (only 2 OTUs - representing 3.5% of total sequences- were shared between the 4 defined groups). Ubiquitous OTUs were affiliated to Soil Crenarchaeotic Group, with high sequence similarities to *Candidatus Nitrospira gargensis*, a moderate thermophilic ammonia-oxidizing *Thaumarchaeota*. Low-salinity clone libraries were dominated by sequences affiliated to Crenarchaeota (41.2% of which 84.7% belonged to the *Miscellaneous Crenarchaeotic Group*) and *Thaumarchaeota* (28.0%). In turn, *Euryarchaeota* dominated in libraries from the Ruppia-rhizosphere samples, ca. 97% of which belonged to *Halobacteriales*. This group represented only the 40% in high salinity sediments. Among the environmental parameters analysed, pH ($R^2 = 0.181$, $p<0.05$) and conductivity ($R^2 = 0.275$, $p<0.01$) showed the higher influence on the distribution of the archaeal communities. Furthermore, UniFrac metrics and principal coordinate analysis revealed a significant influence of macrophytes on the selection of certain archaeal lineages. This effect was more pronounced in euhaline rather than in oligohaline environments.

All together, our results showed that the main factors driving archaeal community composition in coastal wetlands were salinity and macrophytes. Cosmopolitan lineages were rare but they were mainly related to archaeal ammonia oxidizers, which could contribute to nitrification on the studied wetlands. Methanogenic archaea and *Halobacteriales* were also identified as dominant lineages in oligo- and euhaline wetlands, respectively.

**026B Inter-domain horizontal gene transfer, a major role in the adaptation of archaea to mesophilic lifestyles?**

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Twenty years ago, the discovery of widespread marine planktonic archaea via their 16S rRNA genes in environmental samples abolished the traditional view that all archaea were extremophiles thriving in, often anoxic, hot, acid/alkaline or hypersaline environments. Previously, mesophilic archaea (Halobacteriales and some methanogens) were exclusively known within the Euryarchaeota. The newly discovered marine archaeal sequences fell into two different major groups: Group I Crenarchaeota, which was recently proposed to form an independent phylum, the Thaumarchaeota, and the Groups II/III Euryarchaeota. Thaumarchaeota have attracted much attention because of their ubiquitous presence in oceans and soils and the discovery that many of its members are ammonia oxidizers, thus contributing essentially to the N cycle. Despite their importance, only very few Thaumarchaeota have been isolated in pure culture and the information about their genome content is still limited. Marine Groups II and III are much more enigmatic and no member of these groups is available in culture. Metagenomic analyses are therefore appropriate tools to get access to the genes and genomes of these organisms. Phylogenetic analyses of fosmid ends and individual genes in 16S rRNA-gene-containing fosmids from deep-sea metagenomic libraries suggested a high level of horizontal gene transfer, mostly from bacteria. This appears to be further confirmed by the recent assembly of a consensus genome for Group II archaea from metagenomic data. Using more extensive sequencing of deep-sea metagenomic libraries, we show that inter-domain horizontal gene transfer is an ongoing process in marine archaea. Many of the transferred genes correspond to energy metabolism and metabolite transport across membranes, suggesting that horizontal gene transfer
plays an important role in the environmental adaptation of these marine archaea. A preferential bacteria-to-archaea gene transfer has been also observed in halophilic archaea, which allows hypothesizing that the acquisition of foreign genes may have facilitated the independent adaptation of different archaean phyla to mesophilic lifestyles.

027B  Archaeal abundance and diversity in tidal flats, Northern Arabian Gulf
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Tidal flats are productive marine ecosystems that occupy a strategic logistic position as intermediate zones where the aquatic system meets the terrestrial environment. Therefore, there are great expectations that this environment harbor unique microbial groups. Among the least studied group of microbes in the tidal flats are the archaea thus; the current study focuses the attention to the archaeal population inhabiting tidal flat sediments Northern Arabian Gulf. During the study, archaeal diversity and abundance was investigated in Kuwaiti bay and non-bay stations during summer and autumn. Sediment samples from the tidal flats were collected using core sampler and the total number of archaea was determined using fluorescent in situ hybridization (FISH). In addition, the cultivable archaeal population was investigated using CDM and MGM media and the purified isolates were identified molecularly using 16S rRNA gene sequencing. The results showed that Haloferax sp., Halogeometricum sp. and Natrinema sp. dominated the culturable archaea while the Halorhabdus sp., Halomicrobium sp. and Halorussus sp. dominated the unculturable archaea based on denaturant gradient gel-sequencing technique. In addition, the FISH results showed that the total numbers of archaea were higher in summer than in autumn and in the muddy (M) sediment layer. On the other hand, the sandy layer (S) has less numbers of archaea for all sediment zones. Also, the bay stations were richer with archaea than the non-bay station. In conclusion, temporal and spatial variation in archaeal distribution was detected in the tidal flats Northern Arabian Gulf.

028B  Archaea in canopy wetlands
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Ground wetlands are the main natural source of methane but they fail to explain the observed amounts of methane over tropical forests. Bromeliad tanks are discrete habitats for aquatic organisms and up to several thousand of bromeliad individuals per hectare of tropical forest create a unique canopy wetland ecosystem in neotropical forests. Recently, we have discovered that canopy wetlands inhabit methanogenic archaea, emit substantial amounts of methane and may help to explain the high amounts of methane over neotropical forests. However, the distribution of the archaeal community composition and methane production in canopy wetlands of different tropical forest ecosystems have not been studied, yet.

In this study, we investigated the archaeal communities and methane production of bromeliad tanks along an elevation gradient in neotropical forests for the first time. We distinguished three functional types of tank bromeliads, based on plant architecture and ecological niche preference. We sampled the tank substrate of 10 tank bromeliads per functional type and elevation (1000 m, 2000 m and 3000 m above the see level). Functional type I-tank bromeliads are concentrated in the understory and on the ground. Functional type II and type III are concentrated in the mid and overstory. Molecular analyses were performed based on the archaeal 16S rRNA via terminal restriction fragment length polymorphism and methane production potential was measured by tank-substrate incubation experiments.

Multivariate analyses revealed different community patterns and different methane production potentials between the different elevations and between the functional bromeliad types at each elevation. In contrast, tree and bromeliad species composition at each elevation had a much lower effect on archaeal community composition and methane production potential. The terminal restriction fragment size of 393 base pair length, which is characteristic for methane producing Methanocellales (Rice cluster I), was dominating in bromeliads tanks across elevations and functional bromeliad types. However, this dominance decreased with decreasing canopy height at each elevation. The methane production potential of bromeliad tanks was highest at the 1000 m elevation and increased with increasing canopy height at each elevation.

Our results provide novel insights into the spatial distribution of archaea and methane production in neotropical canopy wetlands and suggest that canopy height is a more important indicator than tree or
bromeliad species diversity on how archaeal community composition, methanogenic pathway and methane production changes within canopy wetlands.

**029B**  
**Photoinhibition of archaeal and bacterial ammonia-oxidizers: insights from laboratory cultures and natural stream biofilms**  
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It has been known for decades that light is an important inhibitory factor of ammonia oxidation. Inhibition by light potentially influences the distribution of ammonia oxidizers in aquatic environments and is one explanation for nitrite maxima near the base of the euphotic zone of oceanic waters. Previous studies of photoinhibition have been restricted to bacterial ammonia oxidizers, whereas studies for archaeal ammonia oxidizers, which dominate in many aquatic and terrestrial environments, are scarce. We present an overarching study to evaluate the effect of light on ammonia oxidizing archaea (AOA) and bacteria (AOB) merging a culture approach and community characterization of natural stream biofilm. In laboratory, we observed greater photoinhibition of the AOA in Nitrosopumilus maritimus and Nitrosotalea devanaterra than of the bacterial strains Nitrosomonas europaea and Nitrosospira multiformis under continuous illumination at different light intensities and under light/dark cycles. In addition, unlike bacterial strains, AOA showed no evidence of recovery during dark phases. Hence, irradiance could probably shape the distribution patterns of both AOB and AOA in nature. To test this hypothesis, we selected biofilms that develop in cobbles from five different streams submitted to high nitrogen loads by waste water treatment plants (WWTP) inputs, and to different light regimes and whole biofilm biomasses. We examined AOA and AOB natural abundances by qPCR, and phylogenetic composition by cloning and sequencing of the amoA gene. The sampling was carried out in winter, when biofilm biomass was low and irradiance high, and in summer with higher biomass and lower irradiance both at the light- and at the dark-exposed cobbles surfaces. We observed substantial differences between culture and natural conditions. Upstream the WWTP, AOB were mostly below detection limits and AOA were abundant and ubiquitous, and related to the Nitrososphaera cluster. Downstream the WWTP, AOB where abundant and ubiquitous and related to Nitrosomonas cluster, and had a significantly lower abundance at the highest irradiance. Conversely, AOA were significantly more abundant in light-exposed biofilms and showed different community composition with a domination by sequences affiliated to the Nitrosotalea and Nitrosopumilus clusters. Overall, these results suggest that in natural mature biofilms additional factors such as shading, nutrients concentration, and oxygen availability may overwrite the photoinhibitory effect on ammonia oxidation, and these factors should be carefully considered for full understanding of the ammonia-oxidizers ecology of both AOA and AOB.

**030B**  
**Methanogenic diversity and activity in a high-temperature biodegraded oil field**  
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Recently, microbial ecological research in petroleum reservoirs is of great interest in regard to the microbial enhanced oil recovery (MEOR). The microbiological and biogeochemical studies have indicated that methanogens are distributed over petroleum reservoirs worldwide and produce methane in situ. However, the microbial methanogenic processes in such environments are still poorly understood. In the present study, we investigated the methanogenic community and activity in a high-temperature petroleum reservoir by a combination use of geochemical analyses, radioisotope tracer experiments, culture-dependent and -independent analyses.

The formation water, crude oil and gas samples were obtained from a commercial oil producing well (depth, 938m; temperature, 54°C) in Yamagata, Japan. Carbon isotopic composition of gas components and saturated hydrocarbon composition of crude oil were determined by GC-C-IRMS and GC-FID, respectively. To measure methanogenic potential, formation water samples were collected in N₂-filled sterilized glass bottles. To determine the methanogenic pathway in the reservoirs, radioisotope tracer technique was applied to microcosms comprising of the formation water and oil with a volume ratio of 100:1. Some portion of formation water was fixed with ethanol and used for total cell counts.
with DAPI staining. Phylogenetic analysis of archaeal and bacterial communities in the formation water was conducted based on 16S rRNA gene clone libraries constructed using archaeal- and bacterial-specific primers. Cell density of culturable methanogens was measured by serial dilution with methanogenic substrates, and the cultured ones were phylogenetically identified by direct sequencing their 16S rRNA genes.

In the geochemical analysis, the predominance of isoprenoid over straight-chain alkanes were observed in this study, indicating that the oil has been partially biodegraded. Since the $^{13}$C enrichment of carbon dioxide was also detected in the gas, the crude oil can be biodegraded via methanogenesis. Indeed, radiotracer experiments using [14C]-labelled bicarbonate and acetate clearly revealed that microbial methanogenesis was active in situ and its pathway was mainly hydrogenotrophic. We also observed the culturable methanogens with the order of $10^3$ cells per ml in the formation water, which is approximately 1% of the total cell count ($3.2x10^5$ cells/ml). Based on 16S rRNA gene sequencing analysis, the methanogens cultured in this study were closely related to *Methanothermobacter thermautotrophicus*, a thermophilic hydrogenotrophic methanogen, with 99% of sequence similarity. In the original formation water, *Methanothermobacter thermautotrophicus* was also predominant and occupied 90% of the archaeal 16S rRNA gene clone library constructed. Bacterial community diversity is also low. Most of the bacterial clones (62% of total clones) were moderately related to *Syntrophus* *gentianae*, an anaerobe syntrophically oxidizing benzoate (94% of sequence similarity). *Syntrophus* spp. have been frequently retrieved from anaerobic hydrocarbon biodegrading microbial communities in the previous studies, suggesting that the organisms may contribute to anaerobic oxidation of organic compounds and supply hydrogen to the methanogen in the oil field.

Taken together with clear geochemical evidence for oil biodegradation, our findings demonstrate that formation water in a high temperature oil field harbors active thermophilic hydrogenotrophic methanogens which may be able to contribute to biodegradation of oil via methanogenesis.

**031B The uncultivated SM1 euryarchaeon- uncovering its mystery**
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In most environmental settings, Archaea (although ubiquitous) seem to be minor components of microbial communities and are predominated by a large, diverse bacterial population. The SM1 Euryarchaeon, however, forms biofilms in the subsurface of sulfidic, cold springs and predominates a very minor bacterial fraction (5-10%) therein. These unique biofilms are delivered in high biomass to the spring outflow and can there easily be harvested.

As soon as the biofilms reach the surface and are in contact with oxygen, the SM1 Euryarchaeon seems to selectively seek interaction with sulfide-oxidizing bacteria to form the so-called “string-of-pearls communities”. In these communities, the bacterial partner and the SM1 Euryarchaeon are present in almost equal abundance, pointing at a “real” partnership and possibly a symbiotic/syntrophic relationship. The filamentous bacteria cover the SM1 Euryarchaeon microcolony and allow therefore growth and persistence even in surface waters.

So far, possible metabolic functions of the SM1 Euryarchaeon remain speculative but may be responsible for the environmental success of this organism. Since the SM1 Euryarchaeon is currently the only known Archaeon to absolutely predominate one certain biotope, combined with its appearance in hot spots in Europe and maybe even beyond, a larger (ecological) role can be assumed.

In order to solve this mystery, we are currently conducting a polyphasic experimental study: Analyzing the bacterial partners in the biofilm by using FISH, SR-FTIR and other methods, sequencing the metagenome of the SM1 Euryarchaeon, as well as analyzing its lipid- and ultrastructure will help to understand the (metabolic) role of this unusual archaeon.

Very recently, a sulfate-reducing activity of the biofilm-associated bacteria has been shown. However, the SM1 Euryarchaeon has not revealed any sulfur-based metabolism, although a sulfate-reducing role in the string-of-pearls community had been predicted. Beyond these new results, we will present
the current status of the project and give insights into the metagenome and lipid structure of the uncultivated, fascinating SM1 Euryarchaeon.

**032B  Archaeal ammonia oxidizers are the predominant players along the salinity gradient of the evaporitic high-altitude wetland, Salar de Huasco - Chile**

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Salar de Huasco is located in the Chilean Altiplano at an altitude of 3800 m. This wetland ecosystem is characterized by extreme physical-chemical conditions; such as a broad diurnal temperature variation, <5°C to 20°C (mainly during wintertime), high total solar radiation, >1000 W m⁻², and a salinity gradient, from fresh water springs to brine waters >40 psu. The wetland is considered nitrogen limited and, thus nitrifying microbes are tightly coupled to ammonium availability mainly derived from nitrogen fixation. Herein, we studied archaeal and bacterial contribution to nitrification through inhibitor assays (Allylthiourea for total ammonia oxidation and GC7 for Archaea only) and gene expression of ammonia monoxygenase along the salinity gradient of the wetland during winter and summertime. High ammonium oxidation rates from 1 to 3 μM d⁻¹ were found in the fresh and brackish portions (< 10 psu) of the wetland, during summertime. Ammonium oxidation rates, between 25-90% attributed to Archaea, were positively correlated with in-situ ammonium concentrations. During summertime ammonium concentrations were significantly higher (>0.3 μM) than wintertime values of 0.1 μM. Fresh organic matter was available during summertime from photoautotrophic organisms, including known cyanobacterial nitrogen fixers in the fresh water springs of the wetland (e.g., Oscillatoria spp.). This along with a smaller diurnal temperature variation (10 – 22 °C), higher total solar radiation (2000 W m⁻²) and a supply of ammonium from surrounding soils as a result of higher precipitation, since Altiplanic rain concentrate only during summertime, could contribute to the enhanced nitrogen recycling in this extreme ecosystem during summertime in contrast to wintertime.

**033B  Influence of Ammonium Concentration on amoA mRNA Levels of Ammonia-Oxidizing Archaea within Sand of an Eelgrass Zone (Shimoda, Japan)**

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Marine ammonia-oxidizing archaea (AOA) have been known to oxidize lower concentration of ammonium in culture media. To evaluate the relationship between ammonium concentration and ammonia monoxygenase alpha subunit (amoA) gene mRNA levels of AOA in natural ecosystems, the effect of ammonium concentration on transcription of amoA gene of AOA was examined by incubating sand from the eelgrass zone in Japan under different concentrations of ammonium chloride (0, 0.5, 2, 7, 14 mM). Furthermore, the sand amended with antibiotic reagents (carbesili or streptomycin) was incubated at 0 mM ammonium chloride. AOA and ammonia-oxidizing bacteria (AOB) abundances were estimated measuring gene copies of amoA with real-time PCR. AOA and AOB amoA mRNAs within sand after 8-day incubation were investigated by PCR and real-time PCR with cDNA from reverse transcription with total RNA extracted from the incubated sand. The abundance of AOA was 20 times higher than that of AOB within in situ sand. Maximum nitrite production rate was observed at 0.5 mM ammonium. No AOA amoA mRNA was detected from the incubated sands at more than 2 mM ammonium. No AOB amoA mRNA was detected at any concentrations of ammonium. These results suggest that concentration of more than 2 mM ammonium inhibits the transcription of amoA gene of AOA within sand at the Eelgrass Zone Shimoda, Japan.
Arctic thaw ponds are becoming more prevalent under a warming climate and the associated melting of permafrost. These systems are generally rich in nutrients and organic matter that if mobilized could contribute to a significant production of greenhouse gases, especially methane. Large gradients in greenhouse gas emissions measured in Arctic ponds suggest that knowledge of the microbial assemblages of methanogens (methane producers) and methanotrophs (methane oxidizers) will aid understanding how these ponds will react in the face of changing climate. To study the methane production cycle in ponds located in the Canadian Arctic (73°09'N 79°59'W), the archaeal and bacterial communities from four thaw ponds were identified using 16S rRNA pyro-sequencing of community DNA. In addition, dissolved and bubbling greenhouse gases from 18 ponds from the same region were quantified by gas chromatography and characterized by their stable isotopic signatures. Forty percent of archaeal sequences from a water sample and 88-95% of the archaeal sequences retrieved from four surface sediments belonged to methanogens. Interestingly, we detected a significant negative relationship between dissolved methane concentrations and the number of sediment methanotroph sequences (r=-0.895, P=0.040), with no significant correlation with methanogens, indicating strong control of methane release by methane consumers. Overall, the 16s rRNA gene sequences from the archaeal methanogens had best matches to hydrogenotrophic methanogens (49-81%) compared to acetotrophic methanogens. However, the greenhouse gas isotopic signatures indicated that only the acetotrophic pathway was likely active at time of sampling, with methane δ¹³C from -40 to -59‰ and δD from -304 to -368‰. Methane oxidation rates varied among the ponds; carbon dioxide δ¹³C ranged from -13 to -37‰. This isotopic signature was also correlated to methanotroph sequence numbers (r=-0.927, P=0.024). The absence of a direct link between the microbial community composition and the methane production pathway, which was suggestive from the isotope signatures, could be explained if hydrogenotrophs were inactive in July when the ponds were sampled, but were more active in spring when acetate would have been limiting. In which case, the methane from hydrogenotrophy would have been released through ebullition earlier in the season (being analyzed).

Ozonation followed by granular activated carbon (GAC) has been widely introduced to drinking water purification plants in Japan. Ammonia removal by GAC is of great concern because ammonia can react with chlorine to produce trichloramine, which is undesirable in taste and odor. Extremely low ammonium concentration in drinking water environments may develop a unique community of ammonia-oxidizers. However, abundances and diversity of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) associated with GAC have not been well studied yet. In order to characterize ammonia-oxidizers on GAC and better understand nitrification mechanism of GAC, we evaluated GAC samples from 12 full-scale drinking water purification plants in Japan. In addition, changes in abundances and diversity of AOA and AOB in water along purification process, that is coagulation, sedimentation, ozonation, GAC and sand filtration, were investigated.

The collected GAC samples were used for purification for 4–46 months. Ammonium removal potential of GAC was evaluated by incubating 50 g-wet GAC in inorganic medium containing 1.4 mg N/L of ammonium. Abundances of AOA and AOB were measured by real-time PCR targeting amoA genes. Terminal-restriction fragment length polymorphism (T-RFLP), cloning and sequencing were applied to analyze the diversity of AOA.

Ammonium concentration of raw water in 12 drinking water purification plants were in the range of 3-1.9×10⁵ gene copies/g-dry, while AOB were in the range of 2.7×10⁵ – 2.1×10⁶ gene copies/g-dry. The
ratio of AOA amoA genes to AOB amoA genes was from 0.01 to 621.4. AOA amoA genes were higher than AOB amoA genes for 9 GAC samples. Ammonium removal potential had no positive relationship with the abundance of AOA, AOB or even the sum of them. The specific ammonia oxidizing activity may be highly variable among species of ammonia oxidizers and among samples even for a species. T-RFLP analysis of AOA amoA genes exhibited four operational taxonomic units (OTU) for GAC samples. They were all closely related to *Nitrosopumilus maritimus*. Water samples taken after each step of purification process were analysed for one of the plants. Raw water had $1.7 \times 10^2$ gene copies/mL of AOA amoA genes and $4.6 \times 10^2$ gene copies/mL of AOB amoA genes. While concentration of AOA amoA genes were maintained until ozonation, AOB amoA genes decreased to $2.4 \times 10^1$ gene copies/mL by coagulation and ozonation. Selective removal of AOB in advance of GAC filter provided the clue for AOA predominance on GAC. Increase of AOA in GAC effluent was observed, reflecting dominant proliferation of AOA in GAC filter. Though 8 OTUs were observed in raw water, 2 OTUs which were consistent with OTUs on GAC remained after ozonation. It means selected AOA could survive the purification process and reach to GAC.

036B  **Comparative genomic analysis of the transport systems in Thaumarchaeota**

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A large proportion of the archaea belonging to the Thaumarchaeota are thought to be ammonia oxidizers. Several studies have provided convincing evidence that ammonia oxidizing archaea (AOA) grow autotrophically using carbon dioxide or bicarbonate as their main carbon source. However, there is experimental evidence that AOA in aquatic and terrestrial environments can also take up and possibly assimilate organic substrates. Furthermore, growth experiments with pure cultures of *Nitrososphaera viennensis* indicated that growth of some AOA might even be dependent on the supply of organics.

In order to explore substrate uptake capabilities in the Thaumarchaeota, we currently perform a comprehensive genomic analysis of all transport systems encoded in eight thaumarchaeal genomes, i.e. 6 from the *Nitrosopumilus* cluster/1.1a group and 2 from the *Nitrososphaera* cluster/1.1b group. We attempt to define both, a potential core-set of transporters in the Thaumarchaeota and also unique features in the transporter complement of individual thaumarchaea. For example, all thaumarchaea considered in this study may be able to take up glycerol and sulfonates, respectively, and possess in addition transporters of the drug/metabolite transporter family. Conversely, genes for phosphonate transporters are uniquely present in *C. symbiosum* and *N. maritimus* while those for transporters of the arsenical resistance-3 family are solely represented in *Nitrososphaera* genomes. Substrate uptake and usage by *N. viennensis* was tested using growth stimulation experiments. These tests showed that taurine, a sulfonate, stimulates the growth of *N. viennensis* only in the presence of ammonia and pyruvate but has no effect when pyruvate is absent, further confirming the crucial role of pyruvate in growing cells of *N. viennensis*.

037B  **Determination of the Synergistic Acute Effects of Antibiotics on Acetoclastic Methanogens**

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Antibiotics are one of the most widely used pharmaceuticals. Substantial amounts of antibiotics are released in environment because of the usage in medicine, farming, aquaculture etc and the production in the industry. Pharmaceutical industry has the major importance on the emissions of antibiotics into the environment because of the high level of active compound concentrations up to 1000 mg/L. Effluents from the pharmaceutical industries are characterized by high COD concentration. Thus, anaerobic biological systems could be an alternative to treat the pharmaceutical wastewaters. However these compounds could affect the microbial community structure in biological systems. They are present as multi-component mixtures in environmental compartments. Mixtures generally cause higher effects than individual compounds. In this study, the synergistic short-term effects of Sulfamethoxazole, Erythromycin and Tetracycline, which are widely used antibiotics, on acetoclastic methanogenic activity were determined. In this scope, the acute tests were designed based on a two-
way factorial design where one factor was the composition of antibiotic mixtures, another factor was concentration of antibiotics added (1-250 mg/L). Archaeal 16S rDNA gene clone library was constructed for characterization of seed sludge. All clones were screened by HRM analysis. 22 different OTUs were found. It was determined that Methanosarcinales (27%) is the most abundant classified phylum of archaeal clone library of the seed sludge which includes Methanosarcina and Methanosaeta, two major genera of acetoclastic methanogens. The members of unclassified archaea, Euryarchaeota and Methanomicrobiales were distributed as 58%, 8% and 7%, respectively. The highest inhibition effect of antibiotics on total methane production was observed at 250 mg/L SMX and TET including mixture. The corresponding EC50 values of mixtures were calculated lower than single compounds. Synergistic effects were observed in mixture on acetoclastic methanogens. As a conclusion, mixtures had higher inhibition effect on the total methane production of acetoclastic methanogens than single compounds.

038B Metagenomic analysis of sedimentary ammonia-oxidizing archaea co-cultured with sulfur-oxidizing bacteria
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Ammonia-oxidizing archaea (AOA) are ubiquitous and abundant in marine and terrestrial environments and contribute significantly to global carbon and nitrogen cycles. In this study, we analyzed metagenomes of two sedimentary AOA enriched using sulfur-oxidizing bacteria. We obtained 536.8 and 308.2 Mb sequences from the AOA cultures (AR and SJ culture, respectively). 16S rRNA gene sequence composition of raw reads and tetrancleotide frequency, and GC content of contigs indicate that the enrichment cultures were dominated by three microorganisms; Archaea, Epsilon-proteobacteria, and Gamma-proteobacteria. Two distinct archaeal contigs binned, designated here as "strains AR1 (=SJ) and AR2", showed 80% average nucleotide identity (ANI) each other and showed ANI of 85.2 and 79.5% to N. maritimus, respectively. Genes of central carbon and nitrogen metabolism and their organizations between sedimentary AOA and N. maritimus were well conserved. Besides, 20.5 and 24.4% of total genes were unique and specific to AR1 and AR2, respectively. This difference in gene repertoire might be reflected in the difference in genes involved in the synthesis of cell surface structures and depletion of high affinity phosphate uptake system and many copper-containing respiratory proteins in sedimentary AOA. Intriguingly, further differentiation of AR1 and AR2 were obvious: for example, a set of genes involved in urea utilization and unique restriction and modification system were present only in the AR2 genome. Analysis of bacterial genomes suggests that bacterial sulfur, nitrogen, and carbon metabolism might potentially be involved in interaction with AOA and thus stimulation of nitrification. Our metagenomic results of AOA enrichment cultures provide fundamental information of physiological and metabolic potential of sedimentary AOA and give insight on the strategies for genomic diversification of AOA for niche specialization.

039B AmoA-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions
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Ammonia-oxidizing archaea (AOA) play an important role in nitrification and many studies exploit their amoA genes as marker for their diversity and abundance. We present an archaeal amoA consensus phylogeny based on all publicly available sequences (status June 2010) and provide evidence for the diversification of AOA into four previously recognized clusters and one newly identified major cluster. These clusters, for which we suggest a new nomenclature, harbored 83 AOA species-level OTU (using an inferred species threshold of 85% amoA identity). 454 pyrosequencing of amoA amplicons from 16 soils sampled in Austria, Costa Rica, Greenland, and Namibia revealed that only 2% of retrieved sequences had no database representative on the species-level and represented 30–37 additional species-level OTUs. With the exception of an acidic soil from which mostly amoA amplicons of the Nitrosotalea cluster were retrieved, all soils were dominated by amoA amplicons from the Nitrososphaera cluster (also called group I.1b), indicating that the previously reported AOA from the Nitrosopumilus cluster (also called group I.1a) are absent or represent minor populations in soils. AOA
richness estimates on the species level ranged from 8–83 co-existing AOAs per soil. Presence/absence of amoA OTUs (97% identity level) correlated with geographic location, indicating that besides contemporary environmental conditions also dispersal limitation across different continents and/or historical environmental conditions might influence AOA biogeography in soils.

**040B Novel methylotrophic methanogenic archaea implicated in reduced methane emissions from bovine rumen**
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Enteric fermentation by ruminants accounts for 37% of anthropogenic CH₄ emission to the atmosphere. This CH₄ is derived from a complex anaerobic degradation pathway of plant biomass by the ruminal microbiota, the terminal group being methanogenic archaea. These methanogens are the targets of a plethora of methane mitigation strategies, like feed supplementation, which often aim at reducing H₂ concentrations, as this is the major energy source of all rumen methanogens known to date. However, not all rumen methanogens are yet physiologically characterised.

Dietary rapeseed oil (RSO)-supplementation resulted in a CH₄ reduction of 5.8% in in vivo feeding experiments with lactating Holstein cows. Metatranscriptomics applied on rumen fluid using deep Illumina-sequencing revealed that archaea were significantly decreased, whereas few bacterial and eukaryotic taxa shifted in abundance. The decrease of archaea could be assigned to a novel group of methanogenic archaea distantly related to Thermoplasmata (“Rumen Cluster C”, RCC, Janssen & Kirs, 2008; AEM 74 (12), 3619-3625). In contrast to the well-known hydrogenotrophic methanogens, the novel methanogens decreased in abundance and activity, as observed by 16S rRNA and methyl-coenzyme M reductase (mcr) transcripts. Remarkably, mRNAs of enzymes involved in methanogenesis from methylamines (MA) were among the most highly abundant archaeal transcripts. Similarly to 16S rRNA and mcr of RCC, these mono-, di- and tri-methylamine-related transcripts were only distantly related to known variants from methanogens, and they concurrently decreased in abundance upon RSO amendment making the RCC methanogens the likely origin of these mRNAs. Thus, the RCC are putative methylotrophic archaea, being the second order of methanogens able to utilize methylamines. It is currently unclear if the novel methanogens utilize H₂ as a substrate or if they grow exclusively on methylated compounds. Interestingly, the high abundance of RCC correlated with higher NH₄⁺ concentrations and more bacterial transcripts of NH₄⁺ assimilation systems. This suggests MA as important nitrogen source in the rumen and a tight coupling of carbon and nitrogen cycles via the RCC methanogens.

Metatranscriptomics enabled the in situ characterization of a novel yet uncultured group of methylotrophic methanogens, which has high potential as target for strategies to mitigate CH₄ emissions from ruminant livestock.

**041B Metagenomic analysis of an Archaea-dominated subsurface biofilm**
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Genome sequencing and analysis of distinct representatives of the Archaea have revealed novel insights into the potential ecological role of this domain and have recently lead to the proposal of two new phyla, the Thaumarchaeota and the Aigarchaeota. In 2006, a very deep-branching Euryarchaeon having a genetic distance of more than 20% based on 16S rRNA gene sequences to the next cultivated type strain was identified to form highly pure biofilms in a sulfidic aquifer. These biofilms seem to be washed up from deeper Earth layers (approximately 25 m depth) and are mainly composed of SM1 euryarchaeal cells with a small proportion of 10% bacteria, which are mainly active sulfate reducers. The cells are embedded in a dense network of archaeal cell appendages called “hami”. In order to take a first glance at the genome of this Archaeon, we performed metagenomic 454-sequencing of the biofilm DNA. After filtering for SM1-related contigs, multiple analysis tools to predict functional RNAs and protein coding regions allowed an initial insight into the genome structure of the SM1 Euryarchaeon. Although only 50% of its genome could be covered in this approach, this
data was used for biochemical pathways analysis with astonishing results. Besides the identification of several marker genes like an archaeal RNA-polymerase and 16S rRNA gene sequences, more than 30 genes could be assigned to one specific metabolic pathway. This initial metagenomic analysis provides a great basis for currently ongoing metagenomics and has profoundly contributed to the understanding of the ecological role of this unique archaeal biofilm.

042B  Low-ammonia niche of ammonia-oxidizing archaea in a municipal wastewater treatment plant
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The first step of nitrification is catalyzed by both ammonia-oxidizing bacteria (AOB) and archaea (AOA), but physicochemical controls on the relative abundance and function of these two groups are not yet fully understood, especially in freshwater environments. This study investigated ammonia-oxidizing populations in nitrifying rotating biological contactors (RBCs) from a full-scale municipal wastewater treatment plant in Ontario, Canada. Individual RBC stages are arranged in series, with nitrification at each stage creating an ammonia gradient along the wastewater flowpath. This RBC system provides a valuable experimental system for testing the hypothesis that ammonia concentration determines the relative abundance of AOA and AOB. The results demonstrate that AOA increased as ammonium decreased across the RBC flowpath, as indicated by qPCR for thaumarchaeal amoA and 16S rRNA genes, and core and intact polar lipid (IPL) derived crenarchaeol abundances. This trend was observed in replicate RBC treatment trains and in three seasons over the course of one year. Overall, there was a negative logarithmic relationship ($R^2=0.51$) between ammonium concentration of the wastewater and the relative abundance of AOA amoA genes. Denaturing gradient gel electrophoresis (DGGE) of thaumarchaeal amoA and 16S rRNA genes revealed that a single AOA population was present in the RBC biofilms. This phytype clustered with sequences derived from activated sludge of wastewater treatment plants, but shared low 16S rRNA and amoA gene homology with existing AOA cultures and enrichments. Subsequent enrichment of this AOA phytype from RBC biofilm confirmed that these organisms are capable of mediating the oxidation of ammonia to nitrite, with bicarbonate as the only supplemented carbon source. These results provide evidence that ammonia availability influences the relative abundances of AOA and AOB, and that AOA are abundant and active in some wastewater treatment systems.

043B  The genome of the ammonia-oxidizing archaeon Nitrososphaera gargensis reveals unexpected metabolic versatility and unique adaptations to an extreme environment
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Ammonia-oxidizing archaea (AOA) of the phylum Thaumarchaeota are a diverse, widespread and functionally important group of microorganisms in many ecosystems. However, our understanding of their biology is still very rudimentary in part because all available genome sequences of this phylum are from members of the Nitrosopumilus cluster or group I.1a.

Here we highlight selected genome features of the complete and functionally annotated genome sequence of Nitrososphaera gargensis that was obtained from an enrichment culture. This organism represents a different evolutionary lineage of AOA - termed the Nitrososphaera cluster or group I.1b - frequently found in high numbers in many terrestrial environments. With its 2.83 Mb the genome is much larger than that of other AOA and is characterized by a high number of (active) IS elements/transposases, genetic islands, gene duplications and a complete CRISPR/Cas defense system.
system, which testify to its dynamic evolution and are consistent with low degree of synteny with other AOA genomes. While encoding the repertoire of conserved enzymes potentially required for archaeal ammonia oxidation, *N. gargensis* can also use urea and possibly cyanate as alternative energy sources. Furthermore, its carbon metabolism is much more flexible at the central pyruvate switch point, allows intracellular storage of polyhydroxybutyrate and might even include an oxidative pentose phosphate pathway. Lateral gene transfer from bacteria and euryarchaeota has probably contributed to this extended metabolic versatility. *N. gargensis* is well adapted to its niche in a thermal spring by encoding several copies of archaeal chaperones and mannosylglycerate as compatible solute and has the genetic ability to respond to environmental changes by signal transduction via a large number of two-component systems, by chemotaxis and flagella-mediated motility and possibly even by gas vacuole formation. These findings extend our perception of thaumarchaeal evolution and physiology and offer many testable hypotheses for future experimental research on these nitrifiers.

**044B  Methane production in hydroelectric reservoirs: methanogenic activity potential in brazilian river**

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One of the main factors associated with climate change is the increase of atmospheric greenhouse gases (GHG) concentration. In the last years, the emissions of GHG, such as methane have increased as a result of anthropogenic activities, like energy supply. In Brazil, a significant portion of power demand is meeting with energy provided by hydroelectric power plants, and the biological process related with GHG emission remains unexplored in sediments of areas designed for new hydroelectric reservoirs. Metabolic activity and diversity of organisms associated with the formation of these gases could change after a reservoir construction, in response to water level and organic matter changes in the flooded area. The diversity and expression of genes related to methane Archaea metabolism is often associated with the substrate chemistry. The aims of this work was measure the potential methane emission in Brazilian river and evaluate changes in methanogenic Archaea communities after incubation in anoxic, flowed and different carbon source conditions.

A sample of sediment was collected in São Marcos River located between Minas Gerais and Goias states (43°29′05″N; 5°11′17″E) (area designed for a new hydroelectric power plant). The methanogenic potential was tested by incubating the sample under flooded conditions (using Zinder medium base) with anoxic gas headspace. Four treatments were carried out, each one with a different carbon source (acetate: 10mM, methanol: 10mM, nomeH2CO3: 20% and nomeH2CO3: 100%) and a fifth one with all carbon sources tested. As control, we used inoculated sediment without carbon source. The methane production was measured during three months and methane concentration was analyzed using a gas chromatograph. After incubations were completed, an aliquot was taken for nucleic acid extraction and DGGE analysis of mcrA gene, using the primers (*mLas* and *mcrA*).

Methane production rates and lag times were significant different among the microcosms. In the treatment with all substrates together and in the methanol one the methane production started 10 and 15 days before acetate and hydrogen microcosm, respectively. At the end of incubation, the methane concentration in the acetate treatment was higher compared with the cultures with one carbon source (0,06 mM). In freshwater environments, methane production is commonly more associated with acetate and then to hydrogen, which is a more readily available substrate. The greater production of methane from acetate microcosm over time confirms the importance of this process in this environment. Methanol is a substrate used exclusively by methanogenics, and thus, might explain the shortest time for the production of methane in the cultures with this carbon source. DGGE profiles indicated that only two groups are presented in the all treatments and controls but there is a difference in the profile samples without incubation. The potential methane emission in the sample was confirmed with de increasing of gas production during time incubation and the amount of methane produced depends of carbon source type. However, this condition does not have a significant effect in the structure of methanogenic *Archaea* communities.
Guaymas Basin, in the Gulf of California (Mexico), contains both hydrothermal (Southern through) and cold seeps (Sonora margin) sediments characterized respectively by steep and low temperature gradients. Hydrothermal sediments have now been well documented. In contrast, microbiology at cold seeps sediments of Guaymas Basin remained unexplored to date. In Sonora margin sediments, the hydrocarbon-rich fluids support the development of chemosynthetic organisms mainly composed of assemblages of tubeworms, and dense microbial mats identified as Beggiatoa spp. In this study, diversity and distribution of Archaea, including anaerobic methanotrophs (ANME) and methanogens, were investigated in cold seep sediments colonized by white microbial mat and tubeworms based on porewater concentration profiles of methane, sulfate, sulfide and 16S-23S ribosomal intergenic sequence analysis. Total and metabolically active archaeal diversity was described using 16S rRNA gene libraries. Archaea, Bacteria, methanogenic and anaerobic methanotropic communities were quantified by real-time PCR with newly designed 16S specific primers. Fixed sediments were also observed in fluorescent in situ hybridization with specific probes. Metabolically active archaeal communities were dominated by diverse lineages of sulfate-dependent anaerobic methane-oxidizers (ANME-1, -2a, b, c and -3). However, ANME groups appear to be distributed vertically throughout the sediment cores according to the sulfate profiles. Indeed, ANME-2c were dominant and metabolically active in sulfate-rich sediments despite that ANME-1 populations increased with depth and strongly dominated sulfate-depleted sediments. As previously described at cold seep sediments, ANME-2 formed mixed-type aggregates with sulfate-reducers bacteria, but ANME-1 and, for the first time, ANME-3 representative methanotrophs formed aggregates without physical association with bacterial partner, suggesting that those cells do not necessarily depend on sulfate-reducer partners. These results are the first report of archaeal diversity and the first evidence that anaerobic oxidation of methane and diverse ANME communities occurred in the cold seeps sediments of the Sonora margin, Guaymas Basin.

Archaee represent one of the dominant groups of marine picoplankton. Among these, Thaumarchaeota are now known to represent a highly diversified phylum present in a wide variety of ecosystems (marine, freshwater, soil and hot environments). These ammonia-oxidizing Archaea (AOA) are often very abundant and are a potentially important component of the global carbon and nitrogen cycles. However, little is known about the interaction and possible competition of AOA with other microbial groups. Previous studies have shown that members of the Thaumarchaeota synthesize glycerol dibiphytanyl glycerol tetraether core lipids (GDGTs) with 0–4 cyclopentane moieties, including an unusual GDGT, crenarchaeol. In living Archaea, these lipids are present as intact polar lipids (IPL) with sugar- and/or phosphate-containing head groups attached to the core lipids. Recent studies suggest that crenarchaeol may be synthesized exclusively by Thaumarchaeota making it a potentially suitable marker to trace AOA.

Here, we analyzed GDGTs in the form of IPLs and core lipids in the water column and sediments of different ecosystems: the oxygen minimum zone (OMZ) of the Arabian Sea, the North Sea (transect from coast to central), and Lake Challa (Mount Kilimanjaro, Kenya). The lipid data was complemented by DNA/RNA analysis of metabolic genes of Thaumarchaeota and other microorganisms such as anammox (anaerobic ammonia oxidizers) and ammonia oxidizing bacteria. By using this combined approach we aimed to test the suitability of specific IPLs to trace AOA, and the potential metabolic interaction of AOA with other microorganisms.
Results derived from the Arabian Sea OMZ water column showed a correlation of the concentration profile of the IPL-crenarchaeol with hexose-phosphohexose (HPH) head group with the expression of specific thaumarchaeotal genes in the upper areas of the OMZ. In contrast, anammox bacteria IPLs and genes were more abundant in the core of the OMZ suggesting a large vertical segregation of the niches and a lack of metabolic competition of AOA and anammox bacteria in this system. In the North Sea, Thaumarchaeota were more abundant and active in both water column and surface sediment in Fall-Winter seasons, and also more in coastal stations. In sediments, the depth niche of Thaumarchaeota overlaps with other groups such as anammox, which may promote competition between them. Finally, for lake Challa a correlation in the abundance of IPL-crenarchaeol with the abundance of thaumarchaeotal marine group 1.1a Nitrosoarchaeum-like and soil group 1.1.b Nitrosoarchaea-like in the chemocline of the lake is observed while in deepest areas of the lake, a higher abundance of IPL-GDGT-0 was associated to Crenarchaeota groups 1.2, 1.3, as well as Euryarchaeota methanogens.

Our results demonstrate that the combination of lipid and nucleic acid-based approaches is effective to evaluate the suitability of intact polar lipids as markers of living & active Thaumarchaeota. In addition, it provides key information for unraveling the diversity, provenance, metabolic competition, and abundance of archaeal lipid producers which can help to improve palaeoenvironmental/palaeoclimatic proxies, and to evaluate the role of archaeal members in global carbon and nitrogen cycles.

047B  Archaea: important dwellers in a dark, oligotrophic karst cave, central China
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Caves can serve as natural windows to the subsurface biosphere due to the darkness and isolation from anthropogenic disturbance. Numerous microbes have been isolated, and phylotypes were detected via molecular technique, which provided valuable information to understand the microbial world and the associated microbially-mediated geochemical processes occurring in caves in the world. However, cave archaea are far less understood to date. In this study we investigate the occurrence of the ammonia oxidation archaea (AOA) in sediments along an intermittent stream in Heshang cave, central China via quantitative PCR of the functional gene amoA in combination with their specific lipid biomarker, crenarchaeol, using LC-MS-MS. Meanwhile 16S rDNA sequencing is exploited to construct the bacterial and archaeal clone libraries and elucidate their abundance. Archaea were found to outnumber bacteria averagely by 57-fold. The AOA amoA genes are 12~15,910 times more abundant than amoA genes of ammonia oxidizing bacteria (AOB), suggesting a potentially important role of these AOA in the cave ecology and nitrogen cycling. The occurrence of AOA in great abundance is further confirmed by the dominance of crenarchaeols in archaeal isoprenoid glycerol dialkyl glycerol tetraethers in this unique ecosystem. Surprisingly, despite the great abundance of AOA, the AOB abundance is positively correlated with (r = 0.787) potential nitrification rate (PNR). This may result from the inhibition of the transcription of AOA amoA genes by alkaline pH. Our data underscore the great abundance in archaea especially the ammonia-oxidizing archaea in the dark, energy-limited karst cave, and shed light on the understanding of nitrogen cycling in subsurface biosphere. The ecological function of archaeal community still merits further study.

048B  Revealing the physiology, function and evolution of important archaeal groups in deep-sea ecosystems
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In various deep-sea environments, archaea are considered as key players in biogeochemical cycles of basic elements (such as carbon, nitrogen and sulfur) throughout geological record. Moreover, because of their extreme habitats, these deep marine archaea are carrying information on the ancient Earth and early life yet to be unveiled. Therefore, they are the treasure candidates to study the deep-origin of life on Earth and the adaptation mechanisms to modern environment on both genetic level and molecular level. With parallel applications of high-pressure bioreactor technology, genetic manipulation technology, single-cell capturing and sequencing technology as well as OMICS-based metabolic potential analysis, our group has successfully revealed certain appealing facts on the physiology, function and evolution of some important archaeal groups in the deep-sea ecosystems. Here, as examples, our recent progress in the systematic investigation and genetic engineering on two groups
of deep-sea archaea will be highlighted: piezophilic Pyrococcus from hydrothermal vents; anaerobic methanothrophic archaea from cold seeps. Based on our experimental results and the geochemical data, we propose that the low-energy capture mechanism is essential for early microbial life on Earth.

**049B Systematic investigation on the environmental adaptation mechanism of the piezophilic and hyperthermophilic archaean *Pyrococcus yayanosii* CH1**

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*Pyrococcus yayanosii* CH1 is the first strict-piezophilic hyperthermophilic archaeon (>120°C, >150 MPa) isolated from a sample collected in deep-sea hydrothermal vent. Survey of the mechanisms of environmental adaptation of CH1 will help to find the limit of life in the aspect of temperature and pressure. A facultative piezo-tolerant mutant of CH1 was obtained by random natural transformation and an E. coli-Pyrococcus shuttle vector was constructed to enable functional genomic studies in CH1. Comparative genome and transcriptom analysis of CH1 and its mutant under different temperature and pressure conditions was attempted. About 164 genes were differentially transcribed in the cultivation at 52 MPa and 15 MPa. Transcription of the whole gene cluster that contains complete formate assimilation pathway was found to be up-regulated 10 fold, suggesting a pivotal role of formate respiration for energy conservation under high hydrostatic pressure and high temperature. Elevated expression of the clustered hypothetical proteins flanking to cell division protein FtsZ drops a hint of their function. An integrated investigation of respiration, cell division and other related metabolic pathways will shed light on the adaptation strategy used in the piezophilic and hyperthermophilic archaeaen *Pyrococcus yayanosii* CH1.