Biogeochemical processes in marine sediments are essential parts of the global carbon cycle and climate. Their dynamics can be directly or indirectly traced back to the degradation of organic matter. This process controls, among others, the flux of organic carbon to the deep biosphere, the size of the methane gas hydrate inventory and the long-term burial of carbon in marine sediments and thus ultimately the global climate. Yet, little is known about the controls on organic matter degradation below the shallow subsurface and its evolution during burial. Observations show that the bulk reactivity of organic matter decreases by more than tenfold for each tenfold increase in age. The low energy flux, the exceedingly slow turnover rates, as well as the remoteness of the deep biosphere complicate the investigation and, in particular, the quantification of organic matter degradation and associated biogeochemical dynamics in marine sediments.

Reaction-transport models (RTMs) are, in combination with field or lab observations, ideal tools to quantitatively resolve those dynamics. Here, we illustrate how RTMs can be used in combination with comprehensive data sets to quantify the kinetics of biogeochemical processes in deeply buried Cretaceous black shale sequences and Pliocene-Pleistocene sediments from the Bering sea over different temporal scales. In addition, RTMs also offer means to bridge a large spectrum of spatial and temporal scales and to quantify the biogeochemical dynamics in the context of a system that evolves over years to millions of years as well as over centimetres to kilometres. We illustrate how a combination of reaction-transport modelling and measurements of methanogenesis rates from different glacial environments can be used to estimate the size and location of the potential methane hydrate reservoir below the Antarctic ice shield. Reaction-transport models (RTMs) are, in combination with field or lab observations, ideal tools to quantitatively resolve those dynamics. Here, we illustrate how RTMs can be used in combination with comprehensive data sets to quantify the kinetics of biogeochemical processes in deeply buried Cretaceous black shale sequences and Pliocene-Pleistocene sediments from the Bering sea over different temporal scales. In addition, RTMs also offer means to bridge a large spectrum of spatial and temporal scales and to quantify the biogeochemical dynamics in the context of a system that evolves over years to millions of years as well as over centimetres to kilometres. We illustrate how measurements of methanogenesis rates from different glacial environments can be combined with an RTM to estimate the size and location of the potential methane hydrate reservoir below the Antarctic ice shield.

Decomposition of organic matter in forest soils is a complex process where both fungi and bacteria participate. The differences between litter and soil reflecting the different chemistry of organic compounds contained within are likely to affect the relative importance of individual bacterial and fungal groups in decomposition. The aim of this study was to identify bacteria and fungi active in decomposition with a special emphasis on cellulose and hemicellulose hydrolysis. To achieve this, characterization of microbial community was done by pyrosequencing of soil DNA and RNA and combined with stable isotope probing and the activity of extracellular enzymes (EEA) was analyzed. In Picea abies forest soil sampled in winter, during the period where decomposition processes dominate, microbial communities of different horizons differed considerably in their species composition. Bacterial communities (>20000 OTUs) were dominated by Acidobacteria, Proteobacteria and Actinomycetes, while fungi (>1000 OTUs) mainly belonged to Ascomycetes and Basidiomycetes. While ectomycorrhizal fungi strongly dominated the DNA pool (>80%), the active community was enriched in saprotrophic fungi. Transcript pool of fungal cellobiohydrolase (exocellulase) cbhl was
found to contain 80 different genes in the H horizon and 150-200 genes in the litter horizon, approximately. Out of the genes present in the metagenome, 40% were transcribed in the L horizon and 25% in the H horizon (Baldrian et al. 2012). Interestingly, some of the most abundant transcripts were produced by fungi with very low abundance in the ecosystem. cbhl genes showed distinct association with either the L or H horizons and indicated that different fungal communities decompose cellulose in different soil depths. The analysis of genes involved in hemicellulose and pectin decomposition also showed that the communities of their producers are highly diverse. Analysis of microbes actively decomposing $^{13}$C-cellulose showed a clear distinction of their community from the total community and also confirmed the fact that cellulose degradation is performed by different microorganisms at different soil depths. The results also show that there are many important cellulose-degrading microorganisms which were earlier not recognized as such including some mycorrhizal fungi and Acidobacteria. Microorganisms with low abundance are likely highly important in decomposition processes. The relative ratio of fungal to bacterial biomass in soils along a pH gradient was found to affect the properties of extracellular enzymes which points at the functional specificity of these two groups (Štursová et al. in press). Bacterial and fungal decomposer communities are highly diverse, specific for particular soil horizon and functionally different.

438A  Linking the abundance of nirK, nirS and nosZ functional genes and nitrate removal using alternative carbon sources in laboratory denitrification bioreactors

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Biological denitrification in soil is the main producer of nitrous oxide (N$_2$O) emissions. Denitrifying soil microbes are capable of reducing nitrate (NO$_3^-$) to nitrite (NO$_2^-$) to N$_2$O and di-nitrogen gas (N$_2$). One third of these denitrifiers possess a truncated functional gene pathway, which may lack the nosZ gene and emit N$_2$O as a final emission product instead of the more benign N$_2$. A carbon rich environment, specific to certain types of carbon sources, has been shown to foster an anaerobic environment, which positively impacts microbial denitrification rates. The present study examined the effect of varying carbon sources in laboratory-scale denitrification bioreactors on NO$_3^-$ removal and also correlated performance with the abundance of the denitrifying microbial consortia possessing the denitrifying functional genes nirK, nirS and nosZ in each bioreactor. The bioreactors comprised either lodgepole pine woodchips (LPW), lodgepole pine needles (LPN), barley straw (BBS), or cardboard (CCB), each mixed with soil in a 1:1 ratio (by volume) and subject to sequentially increasing hydraulic loading rates of 3, 5 and 10 cm d$^{-1}$ for a total operation period of up to 744 days. A reactor containing soil only (CSO) was used as the study control. The abundance of denitrifiers was determined by targeting nirK, nirS, nosZ functional genes and the overall microbial population was determined by targeting bacterial and archaeal 16sRNA genes. Nitrate removal from all bioreactors was > 99.7%, but when pollution swapping was considered, this ranged from 67% for LPW to 95% for the CCB; this was also mirrored in the average nirK/nirS/nosZ gene abundance (CCB, c. 94% (c. 10$^8$); LPN, 75% (c. 10$^7$); BBS, c. 74% (c. 10$^6$/10$^7$); LPW, 70% (c. 10$^5$). Bacterial 16sRNA gene abundance was similar in all reactors including the control (P=0.0362). The abundance of nosZ genes and the genetic potential for N$_2$ emissions varied in all reactors in comparison to the control CSO, BBS (P=0.0051); CCB (P=0.0171); LPN (P= 0.0049) and LPW (P= 0.0008). Interestingly, nirS gene abundance was on average much higher than that of nirK and nosZ in the LPN and LPW reactors compared to the CSO, BBS and CCB reactors, indicating a habitat/carbon source preference for denitrifying organisms. Indeed, the high abundance of nir genes in comparison to the total bacterial abundance indicates the possible denitrifying role of fungal and archaeal organisms, which warrants further investigation. In conclusion, it appears the addition of carbon had a direct impact on denitrifier abundance (CSO-10$^5$/10$^6$; CCB/BBS/LPN/LPW- 10$^7$/10$^8$).

083B  Bacterial activities driving arsenic speciation and solubility in marine sediments

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High concentrations in organic carbon and pollutants are found in harbor and marina sediments. Over 50 million m$^3$ of marine sediments are dredged every year in French maritime and commercial ports, the most part of them being discharged in deeper sea zones. High concentrations of arsenic in marine sediments of the South Marseille littoral (France) and excess of carcinogenic effect level through
mussel consumption have recently been reported. If the marine cycle of arsenic is well described in the water column, only scarce data are available on the phenomena controlling arsenic transport from sediment to water column. The aim of the present work was to evaluate the influence of microbial processes on the mobility and ecotoxicity level in polluted sediment marina (L'Estaque) impacted by metallurgical activities and by the commercial port of Marseille. Arsenic concentration was noticeably high (200-350 mg/kg), arsenite (As\textsuperscript{III}) being the dominant form, and arsenic was the major trace element detected in interstitial water.

Biotic phenomena linked to arsenic mobility were compared in dark aerobic and anaerobic microcosms. Sulfate reduction was favored in dark anaerobic microcosms and induced a dramatic increase of arsenic concentration in the liquid phase, linked to the formation of soluble thio-arsenic complexes also detected in the interstitial water, whereas the bacterial activities in aerobic microcosms resulted in a decrease in arsenic transfer from the sediment to the overlying water. Aerobic light microcosms were used to simulate the exposure to natural light of the sediment surface. Here, a clearly distinct, oxidized, red-brown fine layer appeared at the sediment/water interface. The diversity of aoxB genes involved in the bio-oxidation of As\textsuperscript{III} to the less mobile As\textsuperscript{V} (arsenate) was high and similar in the oxidized layer and the deeper black-colored sediment. Such results gave new insights on bacterial As\textsuperscript{III} oxidation, which is to date only poorly studied in marine environments.

Altogether, the results suggest that aerobic microbial processes reduce the transfer of arsenic from the sediment to the water column. Biogeochemical reactions governing its mobility, including direct ones such as oxidation of As\textsuperscript{III} as well as indirect ones such as sulfate reduction, should thus be considered in the management of sediment dredging operations.

439A Nitrite oxidation in the upper water column and oxygen minimum zone of the eastern tropical North Pacific Ocean
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Nitrogen (N) is an essential nutrient that frequently limits primary production, and its distribution is largely controlled by microorganisms that generate energy via N-based redox reactions. Nitrite plays a central role in microbial N cycling because its intermediate redox state permits either oxidation or reduction; it is therefore utilized by multiple microbial groups that may be biogeochemically coupled, or in competition, with one another. We investigated aerobic nitrite oxidation, and the relationship between nitrite and ammonia oxidation, in the upper water column and oxygen minimum zone (OMZ) of the eastern tropical North Pacific Ocean (ETNP) using isotope tracer rate measurements, quantification of nitrite-oxidizing bacteria via quantitative PCR (QPCR) and pyrosequencing, and expression of nitrite oxidoreductase (\textit{nxr}) genes. Nitrite oxidation rates typically exhibited two subsurface peaks at the 7 stations we sampled: one peak was located below the euphotic zone and beneath ammonia oxidation rate maxima; another was typically located within the OMZ. These rate profiles are similar to those reported in the literature, and while maximum nitrite oxidation rates were typically lower than ammonia oxidation rates, integrated rates were more similar. Based on both QPCR and pyrosequencing, nitrite oxidizer communities were dominated by \textit{Nitrospina} bacteria that numbered up to 9% of bacterial communities as a whole. \textit{Nitrospina} QPCR and pyrosequencing data were correlated and tracked both nitrite oxidation rate profiles and \textit{nxr} expression data. Together our findings indicate that \textit{Nitrospina} play a quantitatively important role in nitrite oxidation and N cycling in the ETNP, and provide new insight into their interactions with other key microbial groups in the ocean.

440A The relative proportion of plant C allocated to mycorrhiza and bacteria regulate soil C sequestration
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Large uncertainties still exists regarding the factors regulating the relative rates of formation and decomposition of soil organic matter, making predictions of soil C sequestration in a future climate difficult. Plants allocate a large fraction of C fixed by photosynthesis belowground, and this fraction seems to increase with increasing temperatures and CO\textsubscript{2} concentrations. Elucidating the extent to which this C gets stabilized and remains in the soil is one of the main tenants of understanding C
sequestration in a changing climate. It is generally assumed that allocation of C into structures with long turnover times, e.g. roots and mycorrhizal hyphae, might facilitates the formation of organic matter. On the contrary, C allocation to bacteria and other fast growing heterotrophic microorganisms is believed to stimulate the decomposition of soil organic matter.

The objective of this experiment was to determine if soil C sequestration increase when plants allocate more C to mycorrhizal fungi compared to bacteria. The hypotheses was tested in an experiment where ponderosa pine (Pinus ponderosa), Sitka spruce (Picea sitchensis) and western hemlock (Tsuga heterophylla) seedlings were grown in the same forest soil in separate boxes for six months. Each box was divided into two compartments separated by a membrane. The first compartment contained mycorrhized plants. The mycorrhizal hyphae, but not the plant roots, could proliferate through the membrane into the second compartment. After six months the belowground allocation of plant C to the microbial community was estimated in a \(^{13}\text{C}\) pulse-chase experiment, after which the experiment was terminated and the soil C content relative to the start of the experiment determined.

The soil C content remained constant in boxes with Sitka spruce and western hemlock, while there was an accumulation of C in boxes with ponderosa pine. There was no difference in soil C content in the compartment with roots and mycorrhizal hyphae compared to the compartment with mycorrhizal hyphae but no roots, suggesting that production of mycorrhizal hyphae rather root production caused the increase in soil C content. However, the accumulation of C in boxes with ponderosa pine could not be explained by to the total incorporation of plant derived \(^{13}\text{C}\) into mycorrhizal fungi. On the other hand, ponderosa pine allocated a larger fraction of the \(^{13}\text{C}\) to mycorrhizal fungi and a smaller fraction to bacteria compared to the other plant species, possibly explaining why C accumulated in boxes with ponderosa pine but not in boxes with Sitka spruce or western hemlock. The findings suggest that soil C sequestration is partly determined by the fraction of belowground plant C inputs that is allocated to mycorrhizal fungi relative to bacteria, rather than the total C allocation to mycorrhizal fungi.

440B  Interactions between recalcitrant and labile organic carbon in streams - Are priming effects important in stream biofilm carbon cycling?
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Inland waters - such as streams, rivers and lakes - are increasingly recognized as important components in the global carbon cycle. Dissolved organic carbon (DOC) in these systems is diverse in structure, origin and reactivity, and a fraction of it is regarded as recalcitrant to microbial degradation. In soils, degradation of recalcitrant carbon is often controlled by the availability of labile carbon sources. This is linked to the priming effect (PE). Mounting evidence suggests that PE is also important in aquatic ecosystems but there are so far very few studies addressing this topic. Biofilms are vital components of aquatic ecosystems. In stream biofilms, heterotrophic bacteria and algae coexist in close proximity, exposing the bacteria to both recalcitrant DOC of terrestrial origin and labile organic carbon from the algae. We hypothesize that this makes stream biofilms hotspots for PE. We used plug-flow bioreactors inoculated with natural stream biofilm bacterial communities to test the potential of a priming effect in aquatic ecosystems. The bioreactors were amended with an isotope-labeled plant extract serving as a model of recalcitrant DOC in streams. Labile carbon sources, in the form of glucose and an algal extract were added to induce PE. Nitrate and phosphate were also added to assess the role of these inorganic nutrients on carbon uptake. Microbial uptake of the different carbon sources was monitored by measuring the concentrations and isotopic ratios of respired CO\(_2\), biomass and DOC. Our results suggest that the priming effect plays a role in stream carbon cycling. However, inorganic nutrients appear to enhance the effect. This study indicates that interactions between labile and recalcitrant carbon sources are important for microbial carbon cycling in aquatic ecosystems.

441A  Isolation of heterotrophic nitrogen fixing bacteria from an oxygen minimum zone in the Baltic Sea
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Cyanobacteria have thus far been considered responsible for biological fixation of gaseous nitrogen (N) in the oceans. Nucleotide sequences originating from non-cyanobacterial diazotrophs seem, however, to dominate marine libraries of nitrogenase reductase genes (nifH). This has, in concert with the discrepancies in the marine N budget, led to an increased interest in non-cyanobacterial N fixation. Due to the oxygen-sensitive nature of the nitrogenase enzyme complex, pelagic hypoxic marine environments are potential hot spots for N fixation by heterotrophic diazotrophs, and diverse diazotrophs have been found in oceanic oxygen minimum zones. Thus far, however, quantitative data on the N fixation potential by heterotrophs are lacking. Hence, cultivation dependent autecological studies are urgently needed in order to estimate the importance of heterotrophic diazotrophs in N cycling. In this study microoxic artificial seawater void of reduced N was used as medium for enrichment of diazotrophic organisms from the central Baltic Sea Proper. Glucose, acetate, fumarate, or lactate was used as carbon substrate, and the medium was inoculated with hypoxic water from the oxycline of the Gotland deep (121 m depth, 1.79 μmol O2 L⁻¹). Following 6 months of incubation nifH gene composition in the enrichment cultures was compared to in situ composition. Furthermore, bacteria were plate-isolated from the enrichment cultures. In general there was a high congruence between sequences from the enrichment cultures and in situ. The majority of the nifH sequences from the enrichment cultures and from the oxycline were affiliated with the nifH sequence Cluster I and most showed high sequence similarity to the Pseudomonadaceae family, including a diazotroph previously isolated from the Baltic Sea. Another clade of Cluster I showed high sequence similarity to the Geobacteraceae family. Several Cluster III sequences were also found, both in the enrichment cultures and in the oxycline. These were distributed in three clades showing high sequence similarity to the Chlorobiaceae, the Desulfovibrionaceae, and the Clostridiaceae families, respectively. The majority of the plate-isolates obtained from the enrichment cultures were confirmed to carry the nifH gene. Our work demonstrates that a representative fraction of the diazotrophic community from a hypoxic marine environment could be enriched in culture. Moreover, a fraction of these could be plate-isolated for subsequent autecological study, which is imperative for a future evaluation of the role of heterotrophic diazotrophs in marine N cycling.

Marine oxygen deficient areas are sites of important microbially mediated transformations within the nitrogen cycle. In the Baltic Sea, suboxic waters (oxygen below 5 μmol L⁻¹) are considered to be a major nitritification zone within the water column. Recent evidence indicates that Archaea, dominated by the brenarchaeotal subcluster GD2 which is related to Candidatus Nitrosopumilus maritimus, and not Bacteria are here the major ammonium oxidizers. However, little is known about how the occurrence of the brenarchaeotal subcluster GD2 is related to actual nitritification rates and chemoautotrophic production. To approach this question, we sampled pelagic redoxclines in February, July and November 2011 in the Baltic Sea and determined nitritification and dark, inorganic carbon fixation rates via ¹⁵N and ¹³C isotope incubations, respectively. The abundance of putative ammonia-oxidizing Crenarchaeota was quantified by 16S rRNA based catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH). Additionally, we spiked water from the nitritification maximum with sulfide to determine the impact of sulfide on nitritification activity. Nitritification was detectable throughout the suboxic zone with maxima of 119-131 nmol L⁻¹ d⁻¹ in depths with oxygen below 8 μmol L⁻¹ and was stable throughout the year. Remarkably, a nitritification potential was detected even in the upper anoxic, sulfidic zone. Crenarchaeotal abundance, accounting for up to 24% of total prokaryotic cell counts, correlated strongly with nitritification rates. The CO₂ fixation in the suboxic zone, however, was with 1.6-19.6 nmol L⁻¹ d⁻¹ rather low when compared to the subjacent anoxic, sulfidic waters. The observed maintained nitritification activity in the sulfidic depth was corroborated by the sulfide spiking experiment. After 24 and 48 hours of sulfide exposure, nitritification activity could be detected at up to one third of the original rate. With increasing concentrations, nitritification was reduced notably until terminated at 18.8 μmol sulfide L⁻¹. Our study indicates that ammonia oxidation in the suboxic zone of the Baltic Sea is mainly driven by Crenarchaeota. Their occurrence also in the anoxic, sulfidic water masses and the maintained nitritification potential point to special adaptations in this habitat with a potentially reduced sensitivity against hydrogen sulfide.
443A  Effect of high pressure CO2 on microbial communities and bacterial activities related to carbon capture and storage
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Carbon capture and storage (CCS) in the subsurface is a novel technology in which CO2 is injected at high pressure as supercritical CO2 (SC-CO2). However, the influence of microorganisms on CO2 storage and, conversely, the effect of CCS on the subsurface microflora are still unknown. Since SC-CO2 is also a sterilizing agent, it is essential to consider its effect on subsurface microorganisms. For this purpose we performed high-pressure experiments in laboratory-controlled conditions to determine the effect of SC-CO2 and gaseous high pressure CO2 on microbial community structure and activities. Produced water samples from an oil field were incubated in pressurized vessels with CO2 or with N2 (control). In order to evaluate the effect of SC-CO2, incubations were performed above and below the critical point (31.1°C and 7.38 MPa), where CO2 will be in its supercritical or gaseous state, respectively. During the incubation microbial CO2 fixation or transformation were determined with a focus on homoacetogenic bacteria and methanogens as well as by MPN counting of acid-producing bacteria (APB) and sulphate-reducing bacteria (SRB). Microbial community composition of the samples was also determined by 454 sequencing and compared between the different conditions. The results show that homoacetogenic and methanogenic activities were affected by high pressure CO2 regardless of phase, while pressurized N2 had no significant effect. The number of SRB detected was also affected by the presence of CO2 regardless of phase. In contrast, a decrease of the MPN for APB was only observed when the samples were incubated with SC-CO2 (P>7.38 MPa, 34°C), but not with pressurized gaseous CO2 (P2 negatively affects subsurface microbes with SC-CO2 having a stronger effect than high pressure gaseous CO2.

443B  Abundance of norB and nosZ genes determined in conventional and no-tillage systems in the cerrado (savannah) of southwest of Brazil
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The composition of the denitrifying microbial communities in soil and its functional diversity may be crucial in regulating denitrifying activity and N2O emissions to the atmosphere. Quantitative PCR assay to determine the nosZ gene encoding the catalytic subunit of N2O reductase and nitrite oxide reductase (norB) that catalyzes oxidation of nitrite to nitrate are frequently used to study genetic diversity of ammonia and nitrite oxidizing nitrifying bacteria. We measured the abundance of norB and nosZ genes in a soybean production system under no-tillage and conventional tillage systems in the Cerrado (savannah) biome, using a cultivation-independent molecular technique. Soil samples were collected from an experimental field design of EMBRAPA-CPAO, Dourados, southwest of Brazil. Soils from an adjacent secondary forest and a cerrado (savannah) vegetation were collected and used to compare with the two soybean cropping systems. Total DNA was extracted from soil samples and used as templates in triplicates for quantitative PCR (qPCR) reactions using the primers cnorBF/cnorBR and nosZ2F/nosZ2R for Bacteria. Analysis of variance was performed using the general linear model SAS. Significance was accepted at a level of probability (P) of <0.05. The nitrous oxide reductase gene (nosZ) varied from 1.58.10^6 to 2.28 .10^6 copies g\(^{-1}\) of dry soil and nitric oxide reductase gene (norB) ranged from 10^4 to 10^5 copies g\(^{-1}\) of dry soil. Collectively, the results indicated that norB and nosZ genes showed increased amounts in soils from the cerrado vegetation. The no-tillage system had lower copies (<1500 copies g\(^{-1}\) of dry soil) of gene norB when compared to the cerrado vegetation. Soybean under no-tillage system resulted in a significant increase of nosZ gene, with data 38 to 45% higher than conventional tillage and forest, respectively. Both soybean cropping systems showed clear alterations, when comparing nosZ or norB genes. The no-tillage soybean system showed a high potential to mitigate the emission of N2O gas production, due to high values determined for nosZ gene. Both nosZ or norB genes showed to be useful for monitoring N-cycle transformations in response to soil management for agricultural purposes.
Physiological shifts in the temperature response of denitrifying and anammox bacterial communities in coastal sediments
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A major sink for marine nitrogen (N) is benthic nitrate reduction, via the microbially-mediated processes of denitrification and anammox (anaerobic ammonium oxidation). Nitrate reduction rates are controlled by environmental factors such as temperature and substrate availability, which affect both the kinetics of nitrate reduction as well as the composition of the underlying microbial communities. Understanding how the activities of microbial communities change in response to temperature and organic matter availability may be important for predicting the effects of global change on biogeochemical processes. Therefore, we measured changes in thermal profiles of denitrification and anammox throughout the year, in sediments collected from two Rhode Island coastal sites that differ in temperature and N- and C-loading regimes: the urban Providence River Estuary, as well as a continental shelf site in Rhode Island Sound. Homogenized sediments were incubated in a temperature gradient thermal block at 31 temperatures between 0 and 60 degrees Celsius. Denitrification and anammox rates were measured at the different temperatures using a modified isotope pairing technique. The resulting profiles of denitrification and anammox rates varied substantially by site and season. Differences were driven more by changes in denitrification than anammox. Differences in denitrification profiles at both sites suggest an effect of both organic matter availability and temperature, which alter thermal profiles not simply by shifting the thermal optimum, but by altering the entire profile shape. As a consequence, there was variability in the temperature range over which Arrhenius rate plots were linear, despite the fact that the temperature dependence over each linear region was similar among seasons. Because thermal profiles reflect the capabilities of microbial communities, these results suggest differences in the denitrifier communities linked to changes in physiological response to temperature between sites and seasons.

Nitrite oxidation under low oxygen conditions
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Oceanic waters characterised by oxygen deficient conditions account for less than 0.1% of the world ocean volume, but support greater than 30% of fixed nitrogen loss via either denitrification or anammox. With both field and modeling observations suggesting the expansion of these low oxygen regions, it is imperative that we trace the supply of substrates for these nitrogen loss processes and understand their environmental constraints, in order to evaluate the overall biogeochemical impact of these regions.

Sampling was undertaken in both the permanent and seasonal oxygen minimum zones of the eastern tropical North Pacific and the South Pacific off central Chile (36°S). Natural abundance nitrogen isotope signatures and short-term incubations at in-situ oxygen levels with 15N-labelled nitrite were used to examine the distribution of, and subsequently the effect of oxygen on nitrite oxidation. In addition at the seasonally oxygen depleted site off central Chile, 15N-labelled nitrite experiments were conducted over experimentally manipulated oxygen conditions (0 to 20µM). These experiments clearly demonstrated the oxygen sensitivity of nitrite oxidation, with calculated rates increasing 4-fold over the range of oxygen concentrations examined but also with active nitrite oxidation at oxygen concentrations in the nanomolar range. Our results establish nitrite oxidation as an important player in the nitrogen cycle of oxygen minimum zones.
Thursday 23 August

446A  Bacterial communities potentially involved in arsenic cycle in groundwaters from Lombardia (Italy)
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Human exposure to arsenic typically occurs through drinking water and the World Health Organization indicates a maximum threshold of 10 μg L⁻¹ in drinking water. The aim of our work was to investigate the relationships between microbial populations and type and composition of groundwaters, in order to understand the origin of arsenic contamination in groundwaters from the Northern part of Italy (Lombardia).

Water samples were collected from ten sites (six wells and four piezometers), chosen from the dataset of the Regional Agency for Health Prevention and Environmental Protection of Lombardia, and based on their different levels of arsenic content. Samples from piezometers showed lower pH values, significantly higher concentrations of total dissolved iron and manganese, and significantly higher values of electrical conductivity than samples from wells. The total arsenic concentration in groundwater samples ranged from 0.7 to 171 μg L⁻¹. Samples from eight out of ten sites exceeded the 10 μg L⁻¹ arsenic threshold (D.Lgs. 31/2001). In all the arsenic polluted samples, arsenite was dominant and the arsenite/arsenate ratio ranged from 4 to 7.

The microbial communities of 6 groundwater samples were determined by denaturing gradient gel electrophoresis and Pyrotag sequencing of 16S rRNA genes amplified from environmental DNA. Betaproteobacteria (retrieved in five samples), Gamma- and Epsilonproteobacteria (in four samples), and Alphaproteobacteria (in three samples) were the most represented classes. Bacterial populations of samples from wells and piezometers were correlated with the oxidation processes of sulfur (genera Sulfuricurvum and Thiobrix), iron (genera Gallionella, Sideroxydans, Thiobacillus and Magnetobacterium), manganese (genus Hyphomicrobium) and nitrite (genus Nitrospira). Reductive processes of sulfur, nitrogen and of methylated compounds were displayed by the presence of genera Desulfovibrio sp., Denitratisoma sp. and of methylobacteria in all the samples, whereas dissimilatory iron reduction was displayed only in a piezometer sample by the presence of Geobacter sp.. Chemolithoautotrophic strains were confirmed to be present in some samples by the amplification of cbbL and cbbM genes, coding for the large subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) Type-I and Type-II, respectively. Bacterial genera described as able to cope with arsenic were present in groundwater samples, although no sequence belonged to known arsenic-metabolizing strain. This can be due either to a natural biodiversity of bacterial communities or to the presence of specific arsenic strains in number below the detection limit. Their presence was indeed evidenced by amplification of genes for arsenate respiratory reductase, ArrA, for arsenite oxidase, AioA (formerly referred to as AroA/AoxB), and for arsenate reductase (ArsC) in groundwater DNA.

The obtained data indicate that chemolithotrophic processes dominate at all the sites. In particular, two different arsenic metabolisms are present in the bacterial communities of these arsenic polluted groundwaters of Lombardia: arsenotrophy, growth coupled to arsenate dissimilatory reduction or autotrophic arsenite oxidation, and arsenic detoxification via cytoplasmatic arsenate reduction. In addition to these, reactions carried out by iron reducing and iron/manganese oxidising bacteria could be involved in the arsenic mobilization/immobilization processes from geological substrates to groundwaters.

446B  Does land use influence nitrogen cycling communities in soil?
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Global nitrogen cycling is driven by a combination of biological and abiotic activity. Anthropogenic inputs including mineral fertiliser applications to soil and deposition from industrial pollution make a significant contribution; agriculture can result in major losses of nitrates in water and emission of nitrous oxides, which are greenhouse gasses. Microbial communities are the main biological drivers for these processes. In addition to providing the major natural route by which nitrogen gas is reduced...
to ammonia, they also degrade organic nitrogenous compounds to simple inorganic forms (mineralisation), oxidize ammonia to nitrate (nitrification) and reduce nitrate to nitrous oxides and nitrogen gas (denitrification). Nitrate is more mobile in soil than ammonia and prone to leaching, as well as being the substrate for denitrification. Nitrification is carried out by relatively few groups of bacteria and, as reported recently, the dominant group of archaea in soil. A broad spectrum of soil bacteria and archaea are capable of denitrification which is an important source of nitrous oxide although complete reduction of nitrate leads to losses of climatically-innocuous nitrogen gas. However, this final step is not always active and not all denitrifiers possess the gene for nitrous oxide reductase (nosZ). To improve the sustainability of food production, it is important to minimise fertiliser losses due to both nitrification and denitrification but ecological factors in soil that influence the activity of nitrogen cycling communities are not well understood, impeding the potential for mitigation by land management.

We assessed the influence of different long-term fertilization and cultivation treatments in a 160-year-old field experiment (the Broadbalk experiment at Rothamsted farm). Soil nitrous oxide emissions (denitrification potentials) and the size and diversity of denitrifier communities were measured. Abundances of the alternative dissimilatory nitrite reductase genes nirK and nirS, together with nosZ, were estimated using quantitative real-time PCR. We also assessed nitrification rates and the abundance of bacterial nitrifiers based on the ammonia monooxygenase gene amoA, and investigated other nitrogen-cycle genes including the nitrogenase gene nifH and the ammonia transporter ammB.

The activity and abundance of bacterial nitrifiers appeared to increase with soil nitrogen applications. The denitrification potential was much higher in soil from an area of regenerated woodland (left to develop from arable cultivation) than from a farmyard manure-fertilized arable treatment, which in turn had a significantly higher potential than inorganic nitrogen-fertilized and unfertilized arable plots. Communities varied in the relative abundance of nirK, nirS and nosZ; however, the significance for nitrous oxide emissions is unclear. The abundance of nirK correlated strongly with the denitrification potential which in turn correlated positively with total soil carbon and nitrogen. There was an inverse relationship between nirK and nirS (which was less abundant in all our soils). However, the relative abundance of nosZ, five-fold lower than nirK and nirS combined, implied limitation of the final step of denitrification leading to nitrous oxide rather than nitrogen emissions.

We conclude that bacteria containing nirK are most probably responsible for the increased denitrification potential associated with nitrogen and organic carbon in this soil.

009A  Interactions of arsenic species with bacterial species in natural waters
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Arsenic (As) contamination of water systems associated with either geochemical sources or with residues derived from industrial and agricultural activities constitute a major public health problem. The two most common inorganic arsenic species to which human populations might be environmentally or occupationally exposed are arsenite As(III) and arsenate As(V). We aimed to improve our knowledge on the biogeochemical processes of As compounds in view of the arsenic removal from aqueous medium. In the current study, arsenic–resistant bacterial species were isolated from Lakes, Rivers, freshwater wells and natural mineral waters from the Latium region (central Italy), in which volcanic formations and aquifers containing As contribute to water contamination. The bacteria capable of either oxidizing As(III) or reducing As(V) belonged, on the basis of the 16S rRNA gene sequence analysis, to the Proteobacteria, Firmicutes and Bacteroidetes. Because taxonomic and phylogenetic information cannot be linked to specific bacterial processes, we examined ecologically important genes, with particular attention to genes codifying the ArsB, an As(III) efflux membrane protein pump related to the arsenic resistance. We also compared arsB of our arsenic–resistant bacterial species with homologous genes obtained from publicly available bacterial genome sequences. Significantly, the findings, in combination with our previous results (Davolos & Pietrangeli, 2011, Chemistry and Ecology, 27, (S1): 1–9), suggest that horizontal gene transfer may be a major source of As resistant bacterial species.
In situ nitrogen fixation is the biological conversion of N₂ gas into NH₃ (also known as diazotrophy) and is the main source of biologically accessible nitrogen to the biosphere. Deep-sea sediments represent an enormous reservoir of microbial cells and metabolic diversity, but little is known about the extent to which they harbor nitrogen fixation. To better understand the role of deep-sea diazotrophy in marine nitrogen cycling as well as local ecosystem processes, we investigated the magnitude and spatial distribution of nitrogen fixation, as well as its physiochemical controls, in 50 sediment samples representing distinct depth horizons from ten sediment cores collected from Hydrate Ridge, OR, USA (550-800 m water depth), and Monterey Canyon, CA, USA (700-2800 m water depth). The sample sites were selected to represent a diverse set of chemical regimes: Hydrate Ridge cold seep sediments receive methane from degassing hydrates below (a source of C for anaerobic methanotrophs), the Monterey Canyon sediments were collected beneath whalefalls (a source of complex organics), and at both sites, additional sediments were collected 50 m to several hundred meters off-site. Using over 400 bottle incubations amended with either ¹⁵N₂ or ¹⁵NH₄, followed by bulk isotope analysis with an Elemental Analyzer Isotope Ratio Mass Spectrometer (EA-IRMS), we observed the magnitude of nitrogen fixation and ammonium uptake (as a proxy for total growth) in these diverse sediments over the course of a year. Nitrogen fixation was detected in all of the cores, and at rates higher than those reported previously in deep-sea sediments. However, nitrogen fixation was not ubiquitous, and instead was limited to discrete depth horizons within each core. The commonly accepted paradigm that nitrogen fixation only occurs when other nitrogen sources are scarce would suggest that nitrogen fixation would be largely regulated by the presence of bioavailable N, such as ammonium. However, the in situ concentration of ammonium in the sediment porewater measured by Ion Chromatography had no discernable relationship with the diazotrophy measured in the incubated sediments. In fact, nitrogen fixation was observed in sediments with up to 500 µM starting NH₄. In a follow-up experiment, we added increasing levels of NH₄ to a series of homogenized sediment incubations and found that nitrogen fixation occurred in the presence of up to 2 mM of NH₄. This indicates that if there is an ammonium shutoff mechanism in these diazotrophs, it is not relevant for typical concentrations of ammonium in deep-sea sediments. Sediments incubated under a headspace of methane often demonstrated enhanced rates of nitrogen fixation, especially from Hydrate Ridge, indicating a potential methane dependence of diazotrophy in these communities. The influence of additional physical, chemical, and biological controls on deep-sea diazotrophy are currently under investigation. In summary, we report nitrogen fixation rates in a diverse set of deep-sea samples, and suggest that diazotrophy by sediment-hosted microorganisms is regulated by a unique and potentially complex set of parameters.

Denitrifying bacteria produce much N₂O at low pH, and we are beginning to understand why
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Denitrification in soil is a major source of atmospheric N₂O, a potent greenhouse gas and ozone-depleting compound. Denitrifying prokaryotes use NO₃⁻ as terminal electron acceptors in response to oxygen depletion. The process emits a mixture of NO, N₂O and N₂, depending on the relative activity of the enzymes catalyzing the stepwise reduction of NO₃⁻ to N₂O and finally to N₂. Soil pH emerges as a master variable determining the microbial community composition as well as its denitrification product ratio (N₂O/N₂). Several studies demonstrate that this ratio is higher in acid than in alkaline soil. It is therefore likely that emissions of N₂O from agro-ecosystems will increase in large parts of the world where soil pH is decreasing due to intensified management and increased use of chemical fertilizers. Considering its immense implications, surprisingly few attempts have been made to unravel the mechanisms involved in the pH-control of the product stoichiometry of denitrification. We investigated the kinetics of gas transformations (O₂, NO, N₂O and N₂) and transcription of functional genes in intact soil samples from long-term liming experiments. Expression of nirS (encoding nitrite reductase) and nosZ (encoding N₂OR) was generally lower at pH6 compared to pH8, but the nosZ
/nirS transcript ratios were similar or even higher at pH6. These results were largely corroborated in refined experiments using Nycodenz-extracted bacteria from the same soils, which allowed us to better control the pH levels experienced by the cells. The findings indicated that the higher N₂O/N₂ product ratios at pH6 were due to a post-transcriptional effect. Experiments with P. denitrificans demonstrated N₂O/N₂ ratios < 10⁻³ at pH 7 while N₂O reduction was severely inhibited by suboptimal pH (~100% N₂O production at pH=6). This inhibition occurred during protein synthesis/assembly rather than at the transcription level since the relative transcription rate of nosZ versus nirS and norB was unaffected by pH, and since low pH had a moderate effect on the N₂OR activity in cells with a denitrification proteome assembled at pH 7. Ongoing investigations of the N₂OR protein structure will show if this hypothesis is correct. The results are important for the understanding of main factors affecting N₂O emissions from terrestrial ecosystems.

449A Nitrite oxidation in the oxygen minimum zones of eastern tropical south Atlantic and Pacific

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Oxygen minimum zones (OMZs) can significantly affect the Ocean's capability to sequester CO₂, as they are responsible for ~30-50% of global loss of oceanic nitrogen (N), an often limiting nutrient to primary production. Although the known N-loss processes, anammox and denitrification, have been intensively studied, suboxic conditions in OMZ waters also permit the concurrence of other aerobic and anaerobic N transformations that would alter the availability of substrates directly linked to N-loss. One example is the rarely measured NO₂⁻ oxidation, the 2nd step of nitrification and the primary source of nitrate in global oceans. Parallel ¹⁵N labeling experiments were conducted to directly determine rates of known N-cycling processes in the eastern tropical south Pacific (ETSP) and the Namibian OMZs. NO₂⁻ oxidation was found to occur throughout both OMZs, with rates up to 930 nM d⁻¹ and 372 nM d⁻¹, respectively. In congruence, known nitrite-oxidizing bacteria (NOB), including Nitrospina and Nitrococcus, were detected throughout as a major component of the microbial community. In comparison, NH₃ oxidation occurred at lower rates between 0.2-49 nM d⁻¹ in the coastal ETSP and 23.6-123 nM d⁻¹ in the Namibian OMZ. Apparently, additional NO₂⁻ sources would have to be present. Indeed, the majority (76% and 63% on average in the ETSP and the Namibian OMZ, respectively) of NO₂⁻ production came from NO₃⁻ reduction in these OMZs, at rates ≤1000 nM d⁻¹ off Peru and Namibia) well exceeding both NH₃ oxidation and anammox. Budget calculations from all NO₂⁻ sources and sinks revealed that the measured NO₂⁻ oxidation rates were sometimes sufficient to oxidize all of the NO₂⁻ produced via NH₃ oxidation and NO₃⁻ reduction combined. Consequently, a significant amount of NO₃⁻ that had been utilized to oxidize organic matter via NO₃⁻ reduction to NO₂⁻ was subsequently recycled to NO₃⁻ by NOB. In other words, NO₂⁻ oxidation in both OMZs is partly decoupled from NH₃ oxidation and hinders a considerable fraction of bioavailable nitrogen from being directly lost via anammox or denitrification. These findings indicate the necessity to take into account nitrification as well as NO₃⁻ reduction to NO₂⁻ in N-balance calculations, in order to fully assess how changing environmental conditions might impact the overall oceanic N cycle at present and in the future.

450A Role of sulfate-reducing bacteria in the reduction of iron in marine intertidal flat sediment

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Iron (hydr)oxides are important electron acceptors in marine sediments. Iron(III) may be reduced microbially by respiratory iron-reducing or fermentative microorganisms. In addition, iron(III) may be reduced with sulfide, that is produced by sulfate-reducing bacteria. However, the relative importance of the different processes in marine sediments, and the microbial community that is involved, are not well known.

In this study, a microbial community that was extracted from non-sulfidic intertidal flat sediment was incubated with amorphous Fe(III) hydroxide under anoxic conditions in semi-synthetic seawater (that
also contained sulfate) supplemented with organic carbon (glucose, amino acids and acetate). The extent of iron reduction as well as sulfur speciation was analysed during incubation. The microbial community was monitored using automated rRNA intergenic spacer analysis (ARISA) and 454 pyrosequencing of whole community genomic DNA was performed.

The metagenomic data showed a prevalence of *Clostridium* species and deltaproteobacterial sulfate-reducing bacteria. Together with the iron and sulfur speciation, this indicates an important role for the indirect reduction of iron via sulfide. Based on our data, a model for carbon, sulfur and iron utilization in this system is presented.

**451A Characterization and quantification of gene transcripts for arsenic respiration and resistance during in situ uranium bioremediation**

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The addition of acetate to anaerobic sediments in Rifle, CO, has been shown to be a promising strategy for in situ bioremediation of uranium. The resulting stimulation of microbial reduction of Fe(III) was also accompanied by an unintended and temporary release of arsenic into the groundwaters, with concentrations reaching more than ten times the limit fixed by the World Health Organization. There are several possibilities for these elevated arsenic concentrations including: 1) microbial reduction of less soluble As(V) to more soluble As(III); 2) the release of arsenic previously adsorbed onto Fe(III) oxides as the Fe(III) is reduced; or 3) abiotic reduction of As(V) with microbially generated Fe(II). However, previous studies have concluded that microorganisms play the defining role in catalyzing the redox transformations that ultimately control the mobility of the metalloid.

To identify the microorganisms that may play a role in the mobilization of arsenic at the Rifle site, we performed a detailed molecular analysis of the diversity of genes associated with arsenic reduction and resistance and evaluated transcript abundance for these genes during an in situ uranium bioremediation field experiment. The microbial populations potentially catalyzing arsenic transformations were identified and quantified by targeting the genes coding for a dissimilatory arsenate respiratory reductase, ARRA, and for an arsenite oxyanion translocation pump, ACR3.

Overall, the majority (> 60 %) of arrA sequences recovered were most closely related to sequences previously described in *Geobacter* species. The most predominant sequences were most similar to *G. uraniireducens* RF4, which was previously isolated from the Rifle site.

Sequences from both ACR3-1 and ACR3-2 subfamilies were recovered. Acr3-1 sequences were most similar to *Geobacter* species, sharing up to 96 and 91% amino acid similarity to *G. uraniireducens* and *G. lovleyi* respectively. More than 65% of the acr3-2 sequences were affiliated with members of the *Betaproteobacteria* class, with the majority of clones (>50%) being most closely related to acr3-2 sequences from the Fe(III)-reducing bacterium, *Rhodoferax ferrireducens*.

Transcripts of arrA and acr3-1 in the subsurface *Geobacter* community were detected when acetate amendments were initiated, stimulating the growth of *Geobacter* species. Transcript abundance rose as acetate availability and arsenic concentrations increased. There was a direct correlation between the number of mRNA transcripts for the arsenic resistance acr3-1 genes associated with *Geobacteraceae* species and increases in arsenic concentrations in the groundwater. In contrast, increases in the abundance of *Geobacter arrA* mRNA transcripts during acetate amendments lagged behind the major release of arsenic.

These results demonstrate that subsurface *Geobacter* species respond to elevated groundwater concentrations of arsenic during in situ uranium bioremediation with increased expression of an arsenic resistance mechanism. Furthermore, the increased availability of acetate as electron donor, and possible enhanced availability of As(V) as an electron acceptor as As(V) is desorbed from Fe(III) oxides, is accompanied by an apparent enhanced capability for As(V) reduction. The molecular
approach described here is likely to be useful for further elucidation of factors controlling arsenic biogeochemistry in a diversity of subsurface environments.

452A Dissolved organic matter remineralization in the Benguela upwelling system
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Dissolved organic matter (DOM) is one of the largest active carbon pools on earth with a size of 700 Pg of carbon, close to the atmospheric carbon pool of 750 Pg. Consequently, any changes in the production or removal processes of DOM in the ocean can have a major effect on the global carbon budget. Previous studies from the marine environment have shown high bacterial assimilation and dissimilation of single and chemically well-defined components like individual sugars or amino acids, but so far little is known about the uptake of complex natural DOM mixtures. Therefore we carried out incubation experiments using a $^{13}$C labelled algal extract as the DOM source, composed of a broad range of thousands of different organic molecules, which is more representative for DOM released by the deterioration of an algal bloom.

The Benguela upwelling region off the coast of Namibia is characterized by some of the highest primary productivity rates in the ocean. Because the northern Benguela system is an oxygen minimum zone (OMZ) and the southern part of the Benguela upwelling system is mainly oxic, it is an ideal system to study the remineralization of DOM under both aerobic and anaerobic conditions. Preliminary results reveal complete remineralization rates of DOM into dissolved inorganic carbon (DIC) of up to $\sim$10 µM C d$^{-1}$, suggesting that a substantial part of the newly fixed carbon can be rapidly remineralized, with similar rates in surface waters (0-20 m) as in the deeper waters (20-120 m).

More intriguingly, the rates of DOM remineralization into DIC were equally high in the anaerobic waters of the northern Benguela as in the aerobic shelf waters in the southern Benguela. In the absence of oxygen and adequate nitrate reduction, this is raising the question of potential oxidants of DOM for DIC production in OMZ waters.

452B Assessment of PCR Primer Specificity in Detecting Anaerobic Ammonium Oxidizing (Anammox) Bacteria and Their Diversity in Different Environmental Samples
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Anaerobic ammonium-oxidizing (anammox) bacteria grow using the energy derived from conversion of ammonium and nitrite into dinitrogen gas in the absence of oxygen, with nitrite as terminal electron acceptor. As one of the latest additions to the biogeochemical nitrogen cycle, anammox bacteria have been studied extensively. Since no pure cultured anammox bacteria are available, culture independent methods have been developed to detect amount, diversity and distribution of anammox bacteria in various ecosystems. PCR amplification of anammox bacterial 16S rRNA and functional genes are commonly used for detection and identification of anammox bacteria. In this study, seven published PCR primer sets for detecting the 16S rRNA and hydrazine oxidoreductase encoding genes of anammox bacteria were compared with four samples from different ecosystems. Most of the primer sets, including three sets for amplifying 16S rRNA and four sets for hzo gene showed high specificity in detecting anammox bacteria from wastewater (WW), Mai Po wetland (MP) and the South China Sea (SCS) samples. But, no positive results were obtained from a rice paddy Baijiang soil with primer sets AMX, H4F, ANA, or CL2. Comparing the different primer sets, no significant differences in specificity between primers sets AMX, BS and Brod could be differentiated. Primer set BS showed relatively higher efficiency than AMX and Brod for MP and SCS. Considering primer sets for amplifying hzo gene, CL1 is the most effective one and H4F and ANA shared similar characteristics. CL2 only worked well for wastewater samples. UniFrac principal coordinates analysis (PCoA) suggested most of the 16S rRNA and hzo gene clone libraries could be grouped separately in terms of sample site except for Baijiang soil. Based on the phylogenetic analyses, sequences were grouped into relevant clusters and new clusters showing lower similarity to the known sequences. Among the four samples, WW indicates the lowest diversity with Candidatus Kuenenia as the dominant species while C.Scalindua accounts for most anammox bacteria in the SCS sample. MP and Baijiang soil share relatively higher diversity covering most of the known anammox bacteria species. Correlations of the anammox
bacterial assemblages with environmental parameters analyzed by canonical correspondence analysis (CCA) indicated that ammonia has a strong relationship with C. Kuvenenia in some environmental samples while the concentrations of nitrite and nitrate contribute to the distribution of anammox bacteria clusters. Results collectively indicate a broad distribution of anammox bacteria with high niche-specific community structures within various ecosystems.

453A Dynamics of anoxic nitrogen cycling pathways and anammox-specific biomarkers in an experimental marine sediment system
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Anammox (anaerobic ammonium oxidation) is an alternative pathway of fixed nitrogen removal to denitrification in aquatic ecosystems, and may represent a significant, previously unrecognized sink in the world’s oceans. The controls on the relative importance of anammox and denitrification are poorly understood, though nitrate and organic matter are both likely factors. We conducted a laboratory experiment using marine sediments collected off the coast of Rhode Island, USA, wherein we manipulated both nitrate and sediment organic matter loadings in a crossed factorial design. We hypothesized that anammox would be favored under conditions when nitrate delivery rates were high relative to organic matter loading. We placed thin discs (1.5 mm x 10 cm diameter) of sediment into large flow-through incubation chambers filled with anoxic seawater. Nitrate levels achieved steady state concentrations of approximately 2 and 20 uM in the low and high loading treatments, respectively, and organic matter additions were achieved using freeze-dried Chlorella algae to increase sediment organic carbon content from 1% (ambient) to 2%. Over a six-week period, we measured potential rates of anammox and denitrification using incubations of homogenized sediments in vials with added 15N-labeled nitrite. In thin discs with added Chlorella, potential anammox and denitrification rates were initially inhibited, while nitrite was rapidly consumed, suggesting high rates of dissimilatory nitrate reduction to ammonium (DNRA). After the labile organic carbon pool was depleted, as inferred from leveling off of ammonium production rates in the tanks, both anammox and denitrification resumed, with higher rates of denitrification than anammox. Both anammox and denitrification rates were higher in the treatments with elevated nitrate concentrations, with highest rates in treatments with high nitrate and high organic carbon, relative to the controls. As hypothesized, the percent anammox of total N2 production (%ra) was highest in treatments with high nitrate but low carbon. The results suggest strong coupling between denitrification and anammox rates, even in sediments receiving a large pulse of readily available organic matter. We also measured concentrations of ladderane lipids, biomarkers for anammox bacteria, in an effort to directly link estimates of anammox bacterial biomass and anammox rates. By measuring these rates and biomarkers in response to our experimental treatments, our approach provides a mechanistic framework for elucidating environmental controls on anammox in marine systems.

454A Microbial extracellular enzyme activity promotes slower cycling of soil carbon in perennial agroecosystems
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There is increasing interest to adopt agricultural management practices that optimize microbial services such as nitrogen retention and carbon storage. Understanding the microbial mechanisms that regulate these benefits could improve ecosystem models and help inform land management decisions. The aim of this study was to evaluate the response of microbial activity to alternative agricultural systems with perennial cover and cover crops. Decomposition models based on natural systems suggest that an increase in belowground carbon inputs associated with alternative systems will stimulate microbial activity and result in a priming of more recalcitrant carbon from soil and root litter. Alternatively, we hypothesize that the greater abundance of soluble root exudates in alternative compared to conventional systems will lower enzyme activity if these plant-derived labile carbon inputs support a greater proportion of the microbial community.

To test for land management effects on microbially mediated carbon transformations, we measured potential and biomass-specific extracellular enzyme activity via fluorometric assays and chloroform-fumigation extraction, as well as potential carbon mineralization rates. Soil was sampled from the
Landscape Biomass Project in Iowa, USA in 2010 and 2011. At this site, plots of continuous corn (Zea mays, control plots), a perennial switchgrass (Panicum virgatum) monoculture, and a double crop of sorghum (Sorghum bicolor) and triticale (xTriticosecale) are grown on five landscape positions in a full factorial complete block design (n=3).

Cellulolytic enzyme activity was lower in soils under switchgrass compared to the annual crops (P=0.02). Similarly, although not statistically significant, switchgrass microbial communities generated less cellobiose (beta-glucosidase) and hemicellulose (beta-xylosidase) degrading enzymes compared to microbial communities in annual systems. Downregulation of cellulose and hemicellulose degradation by microbial communities under switchgrass was suggested by low biomass-specific cellobiohydrolase and beta-xylosidase activity (P=0.02). Despite the reduction in potential enzyme activity, carbon mineralization potential at peak biomass in July was almost twice as high under switchgrass compared to the annual systems (P<0.001).

For perennial agroecosystems, our results support the alternative hypothesis that microbial activity is associated with labile carbon pools. Further, this activity does not result in a greater production of enzymes responsible for the breakdown of more complex forms of carbon, thereby slowing the turnover of soil-stable and root biomass-derived carbon pools. These are some of the first data to provide a mechanistic understanding of the important role of microorganisms in alternative agricultural ecosystems.

455A Substrate preferences of peatland sulphate reducers - elusive microorganisms mitigating peatland methane emission

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Peatlands are a major source of the greenhouse gas methane and their response to global warming and increasing aerial sulphur pollution is one of the largest unknowns in the upcoming decades to centuries. Although regarded as primarily methanogenic environments, biogeochemical studies revealed a hidden sulphur cycle in peatlands which causes rapid renewal of the small standing pool of sulphate. As a consequence, dissimilatory sulphate reduction, which is thermodynamically favoured relative to fermentative and methanogenic processes, often occurs at rates comparable to marine surface sediments and thus effectively decreases gross methane production in peatlands. Our previous work revealed that a Desulfosporosinus species belonging to the 'rare biosphere' has the potential to substantially contribute to sulphate reduction in a minerotrophic peatland. In addition, analysis of the functional marker genes dsrAB [encoding subunit A and B of the dissimilatory (bi)sulphite reductase] revealed a large diversity of putatively novel peatland sulphate-reducing microorganisms (SRM). To illuminate substrate preferences of peatland SRM, we amended anoxic peat-soil slurries regularly with in situ concentrations of single substrates and sulphate and followed sulphate reduction using the 35S-sulphate radiotracer assay. In short-term incubations (6 days), sulphate reduction was stimulated best with lactate, propionate, and butyrate but not with acetate or formate, whereas in long-term incubations (27 days), sulphate reduction was observed under all substrates tested including controls where only endogenous substrates were available. As expected, methane production was drastically reduced in peat-soil slurries amended with sulphate, independent of the tested substrate. Our data indicate that peatland SRM are in involved in a wide range of carbon degradation pathways, either directly or indirectly via syntrophy, with their specific activity depending on the timing of substrate and sulphate availability. In order to elucidate the individual role of selected SRM in the degradation of the various carbon substrates, we are using specific 16S rRNA (gene) and dsrAB-targeted quantitative PCR assays to monitor their growth and transcriptional response in the peat-soil incubations.
The concentration of atmospheric CO₂ is increasing largely due to human activities, and it is projected to reach 550 ppm by the middle of this century. Although it is well established that elevated CO₂ (eCO₂) stimulates plant growth and primary productivity, the impact of eCO₂ on soil microbial communities remains poorly understood. Some previous studies showed that eCO₂ significantly affected the diversity, composition, structure, function and dynamics of soil microbial communities. However, those studies are largely based on individual FACE (free air CO₂ enrichment) experimental sites or ecosystems. The objectives of this study are (i) to examine if the observed soil microbial community responses are site- or ecosystem-dependent, and (ii) to link the soil microbial community structure with soil properties and ecosystem functioning. Towards those goals, we examined the response of soil microbial communities under eCO₂ at six different experimental sites: (i) BioCON (Biodiversity, CO₂ and Nitrogen) at the Cedar Creek Ecosystem Science Reserve, MN; (ii) PHACE (Prairie Heating and CO₂ enrichment) at Cheyenne, WY; (iii) Duke Forest-Atmosphere Carbon Transfer and Storage (FACTS-I) at Duke, NC; (iv) ORNL FACE at Oak Ridge, TN; (v) SoyFACE, and (vi) MaizeFACE at Urbana-Champaign, IL with (i) and (ii) as grass ecosystems, (iii) and (iv) as forest ecosystems, and (v) and (vi) as agroecosystems. A total of 110 soil samples were taken from surface soils (0-5 cm or 0-15 cm). Soil properties were routinely analyzed, and soil DNA was analyzed by a comprehensive functional gene array (GeoChip 3.0) to measure the relative abundance of key functional genes involved in carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) cycles as well as other functional processes. A variety of statistical approaches were used to detect the effect of eCO₂ and ecosystems/sites on soil microbial communities and their linkages with soil properties. Although the overall functional diversity of soil microbial communities remained unchanged by eCO₂ or site/ecosystem, both ecosystem/site and eCO₂ significantly (p < 0.05) affected the functional structure of soil microbial communities with ecosystems/sites having much stronger influence (~42%) than eCO₂ (~1.2%), and 3.2% for their interaction, which may be due to significantly different soil properties among those six sites. The abundance of genes involved in C fixation (for example Rubisco, Pcc/Acc, CODH), C degradation (for example AmyA, endoglucanase, glucoamylase, Lip), methane metabolism (for example PmoA), N fixation (NifH), nitrification (for example AmoA), denitrification (for example NirS, NirK), P utilization (for example Ppk, Ppx), and S reduction (for example DsrA) generally increased in all five sites except the PHACE site under eCO₂. In addition, such changes in the soil microbial community structure were closely correlated with soil C and N. This study provides new insights into our understanding of soil microbial communities and their feedbacks to terrestrial ecosystems in response to eCO₂, implying that we should more consider differential responses of soil microbial communities among different ecosystems.

Sulfur cycle in sewer networks and its consequences on cementitious materials

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Sewer networks enclose many biological and chemical transformations involving sulfur molecules oxidation leading to biogenic sulfuric acid production. Sewage effluents contain many sulfur species such as sulfates and sulfites which are reduced in anoxic zones by sulfate reducing bacteria (Desulfovibrio sp., Desulfobulbus sp….) into hydrogen sulfide (H₂S). This gas is emitted in the aerial part of the pipe and degrades materials. This deterioration proceeds either directly as H₂S is a corrosive gas, or indirectly through the action of microorganisms that will oxidize H₂S into sulfuric acid. This acid will dissolve the cement matrix and produce expansive products (such as gypsum or ettringite). In the worst case it leads to pipes destruction. This study aims at understanding the deterioration mechanisms occuring in the aerial part of the pipe. A special attention is given to the impact of different cementitious material on microorganisms involved
in the global sulfur cycle (sulfur oxidizing bacteria such as *Starkeya novella*, *Halothiobacillus neapolitanus*, *Thiomonas intermedia* and *Acidithiobacillus thiooxidans*). According to field data, cementitious materials made with Calcium Aluminate Cement (CAC) offer far better performance on site than those made of Ordinary Portland Cement (OPC). This behaviour difference is likely associated with a different microorganism diversity.

This work is divided into three parts. The first one consists in an exposition of CAC and OPC mortars in real sewer networks. This on-site experiment aims to follow the evolution of biodiversity and also to characterize the degradation of cementitious materials. Capillary Electrophoresis - Single Strand Conformation Polymorphism (CE-SSCP) analyses performed on each sample reveals that biodiversity is highly related to cement composition. OPC mortars hosts more numerous and diverse microorganisms than CAC mortars.

The second part is represented by various lab experiments aiming to better describe the different steps involved in the whole mechanism. According to our tests, abiotic oxidation of $H_2S$ into elemental sulfur is highly dependent on materials composition. While elemental sulfur deposition can be seen on OPC mortars, none is observed on CAC mortars. Depending on sulfur source available, bacteria will have various growth rates. We have studied the impact of three sulfur sources ($S_2O_3^{2-}$, $SO_4^{2-}$, $H_2S$) on the growth of sulfur oxidising bacteria. All results obtained are used, in a third part, to design an accelerated experiment that should be standardized to test sewer networks materials.

458A  The microbial seed bank: the effects of desiccation and heat stress on methane oxidizing bacteria

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In seasonally changing environments, methane oxidizing bacteria are exposed to adverse temperatures and drought. Studies on pure cultures have shown that representatives of two phylogenetically and biochemically distinct subgroups (type I, Methylococcaceae; type II: Methylocystaceae) form different resting stages with those of type II being far more resistant. While the significance of a methanotroph seed bank has been well established, we lack information on the sensitivity of resting stages to adverse environmental conditions. In particular, it is unclear (i) how representative pure cultures are for the diverse and uncultivated species in soils and sediments, and (ii) how the resting stages’ different resistance may translate into community shifts. Here, we exposed a paddy soil and two lake sediments to drought combined with either ambient or elevated ($75^\circC$) temperature. Upon re-wetting, we followed methane oxidation and population dynamics with time. Fresh soil and sediments served as a control. To assess the response of the methanotroph community, group-specific qPCR assays and a diagnostic microarray targeting the *pmoA* were used. The *pmoA* encodes for the alpha-subunit on the particulate methane monooxygenase, the key enzyme of most methanotrophs. Consistent throughout all treatments and environments, type II methanotrophs recovered well, even after exposure to $75^\circC$, indicating their ability to form desiccation- and heat-resistant resting stages. Interestingly, some type I methanotrophs, thought to be relatively more sensitive to desiccation and heat, increased after treatment, too. More pronounced in the lake sediments, the recovering community was significantly different. These communities, however, were less diverse than in the control suggesting that repeated exposure to adverse factors will change the seed bank’s composition, too. Methanotrophic activity was not compromised despite of a decreasing diversity. Overall, the methanotroph seed bank showed unexpected resilience not only to desiccation, but also to heat stress.

458B  Genes associated with phosphorus solubilisation by plant growth-promoting rhizobacteria

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Mineral phosphate fertilisers are used to increase agricultural production but a large proportion of phosphate applied to soil is rapidly converted to sparingly soluble mineral precipitates that are unavailable for plant uptake. This results in accumulation of phosphorus in soils. For both agronomic and environmental reasons, it is important to improve the efficiency of use of fertilizer phosphorus. A
range of rhizobacteria have been shown to increase availability of soil phosphorus for plant uptake. The aim of this study is to investigate the genetic basis of phosphorus solubilisation by rhizobacteria. A total of 103 rhizobacteria were isolated from New Zealand pasture and forest soils that differed in phosphorus status. The bacteria were screened in plate and liquid assays to determine their ability to solubilise sparingly soluble hydroxyapatite. The 36 isolates with strong phosphate solubilising activity were identified by 16S rRNA sequencing, with most isolates identified as Pseudomonas spp. Ten isolates were assessed in a glasshouse pot trial using hydroxyapatite as the sole phosphorus source. Some isolates increased growth and phosphorus uptake by perennial ryegrass (Lolium perenne) plants by up to 13 percent compared with the control. To optimize the potential selection of novel genes or pathways involved in phosphorus solubilisation, three isolates from different genera (Burkholderia, Enterobacter, and Pseudomonas) were subjected to transposon mutagenesis and the insertion points were determined by DNA sequencing. A core set of genes encoding enzymes in organic acid production and the chorismate biosynthesis pathway are involved in phosphorus solubilisation. Knowing the DNA sequence of these genes will provide the basis for in situ assessment of gene expression in the rhizosphere. Factors affecting the regulation of phosphorus solubilising genes will be assessed in the rhizosphere of ryegrass under both high and low phosphorus conditions.

459A  Humic acids as accelerators of microbial Fe(II) oxidation
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Biogeochemical cycling of iron and organic matter are tightly coupled as recent findings suggest that up to 22% of organic carbon in marine sediments is bound to reactive Fe(III)-species forming a "rusty carbon sink" that is not accessible for microbial decay. Also in terrestrial soils and freshwater systems, the bioavailability of carbon compounds is reduced by complexation reactions, like adsorption or incorporation, with Fe(III). Regarding the cycling of iron, organic matter can facilitate Fe(III) reduction by e.g. serving as electron shuttle. Dissolved Fe(II) that is complexed in colloids or particles with heterogeneous organic carbon (e.g. humic acids), could impact chemical or microbial processes like Fe(II) oxidation although the underlying mechanisms are poorly understood as previous work has provided conflicting results. Therefore, we elucidated the effects of humic acids on (1) the kinetics of microbial Fe(II) oxidation, (2) the dominant biogenic mineral phases formed, and (3) the formation of Fe-organic matter complexes during oxidation. The microaerophilic, Fe(II)-oxidizer Sideroxydans lithotrophicus strain ES-1 was grown in liquid medium or in gradient tubes in the presence or absence of peatland humic acid extract. In liquid cultures (pH 6.6), Fe-oxidation kinetics were determined by regularly measuring HCl-extractable Fe. In treatments amended with 1% peatland humic acid extract Fe(II) oxidation by ES-1 was 18 µM h⁻¹ during the initial 4 days, which was 2.2 times faster than in unamended live controls (8 µM h⁻¹). Raman spectroscopy and energy dispersive X-ray spectroscopy were applied to identify the minerals forming in Fe-oxidizing cultures and to confirm their elemental composition, respectively. Humic acid addition led to the formation of more fine grained, filamentous, and smaller sized (2-3 mm) Fe(III) minerals compared to unamended live controls, where larger Fe(III) oxides (3-5 mm) dominated. Lepidocrocite was the main mineral in all samples together with small amounts of ferrhydrite and goethite. Energy dispersive X-ray spectroscopy confirmed that elemental composition of minerals were independent of the presence or absence of humic acids. In addition, depth dependent voltammetric measurements of Fe species in gradient tubes performed over the course of microbial Fe(II) oxidation suggested the presence of different chemical species in treatments with and without humic acids compared to chemical controls. Our study provided evidence that humic acids accelerate microbial Fe(II) oxidation, especially during the initial phase of oxidation. This could allow microbial Fe(II) oxidation to outcompete chemical oxidation in the presence of humic acids, which is of major importance for Fe(II)-oxidizers persisting in circumneutral pH environments.

460A  Methanogenic oil degradation in the Dangang oil field
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The microbial degradation of hydrocarbons in oil reservoirs affects the quality and economic value of recovered petroleum products. Recent studies suggest that anaerobic biodegradation may play a
significant role in situ and evidence for the biodegradation of residual oil constituents under methanogenic conditions has been reported (for example Jones et al. 2008). Methane, like other biogenic gases, may aid in oil viscosity reduction and enhance flow characteristics through the reservoir matrix. In addition, methane may be used directly as a downstream energy source.

In light of these situations, the aim of this study was to assess the ability of indigenous microbial communities from a thermophilic oil reservoir (Dagang oilfield, China) to produce methane from crude oil under environmental conditions. The isotopic composition of reservoir fluids (H2O, CO2, CH4) was analyzed, and GC-MS fingerprinting was applied to assess the effects of biodegradation on the oil composition in the reservoir. Bacterial, Archaeal, sulfate-reducing and methanogenic numbers were assessed by qPCR of the 16S rRNA, dsrA and mcrA genes, respectively. In addition, microcosms containing either 13C-labelled n-alkanes or aromatic hydrocarbons were inoculated with production and injection waters from Dagang in order to characterize these processes in vitro.

Analysis of petroleum samples confirmed that the majority of the oils from Dagang are highly weathered, and nearly devoid of n-alkanes, alkylbenzenes, alkyltoluenes, and light PAHs. Changes are clearly evident in the distribution of molecular markers, when comparing degraded and non-degraded samples. Geochemical data from reservoir oil, water and gas are consistent with in situ biogenic methane production linked to aliphatic and aromatic hydrocarbon degradation. The average δ13C for methane was around -47‰ and CO2 was highly enriched in 13C (values up to +17.2‰). The bulk isotopic discrimination (Δδ13C) between methane and CO2 was between 32 and 65‰, and is in accordance with previously reported results for methane formation during hydrocarbon degradation (Feisthauer et al. 2010).

Subsequent degradation experiments revealed that autochthonous microbiota are capable of producing heavy methane from 13C-labelled n-hexadecane or 2-methylnaphthalene, and suggest that further methanogenesis may occur from the aromatic and polyaromatic fractions of Dagang reservoir fluids. Microbial numbers in oil/water samples from production wells were abundant (averaging around 107), and included numbers of methanogenic Archaea in the same order of magnitude. In summary, the investigated areas of the Dagang reservoir may have significant potential for testing the viability of in situ conversion of oil to methane as an enhanced recovery method, and biodegradation of the polyaromatic fractions of the oil may be an important methane source.

461A Insights into the unexplored diversity of the nitrous oxide reducing microbial community
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Nitrous oxide (N2O) is a major radiative forcing and stratospheric ozone depleting gas emitted from terrestrial and aquatic ecosystems. It can be transformed to N2 by bacteria and archaea harboring the nitrous oxide reductase (N2OR), which is the only known N2O sink in the biosphere. Despite its crucial role in mitigating N2O emissions, knowledge of the N2OR in the environment remains limited. Here, we report a comprehensive phylogenetic analysis of the nosZ gene coding the N2OR in genomes retrieved from public databases. The resulting phylogeny revealed two distinct clades of nosZ, with one unaccounted for in studies investigating N2O reducing communities. Examination of N2OR structural elements not considered in the phylogeny revealed that the two clades differ in their signal peptides, indicating differences in the translocation pathway of the N2OR across the membrane. Sequencing of environmental clones of the previously undetected nosZ lineage in various environments showed that it is widespread and diverse. Using quantitative PCR, we demonstrate that this clade was most often at least as abundant as the other, thereby more than doubling the known extent of the overall N2O reducing community in the environment. Furthermore, we observed that the relative abundance of nosZ from either clade varied among habitat types and environmental conditions. Our results indicate a physiological dichotomy in the diversity of N2O reducing microorganisms, which might be of importance for understanding the relationship between the diversity of N2O reducing microorganisms and N2O reduction in different ecosystems.
462A Effect of long term nitrogen amendment on denitrifier community structure and activity along an established gradient of carbon availability
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The community structure and activity of denitrifying microorganisms in managed soil ecosystems are influenced by both nitrogen (N) and carbon (C) availability. In this study, we examined the effects of N fertilization in combination with long-term organic amendments that differ with regard to C availability on the potential activity and community structure of denitrifying organisms. Soils were sampled from the Ultuna long-term organic amendment experiment, in which replicate plots have been treated with straw, sawdust, or peat amendments with and without N fertilization for the past 56 years. Analysis of the community structure of different assemblages of denitrifying microorganisms by terminal restriction length polymorphism (T-RFLP) of nosZ, nirS and nirK genes encoding different reductases in the denitrification pathway revealed a differential response to carbon and nitrogen treatments among denitrifiers harboring the nirS or nirK genes, which are believed to be mutually exclusive in the genomes of denitrifying organisms. By contrast, denitrifier communities with nirK and nosZ genes showed a similar response to soil treatments, with nitrogen addition resulting in more similar community structures across soils with different C availability. Potential denitrification activity was significantly higher in response to nitrogen amendment, outweighing the effect of increased carbon availability. However, amendment with different organic matter also resulted in a pH gradient (4.7-6.3) across treatments, which was significantly correlated to denitrifier community structure and potential activity. Concurrent measurement of the metabolic quotient, determined by calculating the ratio of basal respiration to substrate-induced respiration, showed a strong negative correlation to pH that is indicative of elevated environmental stress in low pH conditions. These results indicate that carbon and nitrogen availability play a more distal role in regulating denitrification by determining community niche among sub-populations of denitrifiers, whereas pH has a more direct influence on potential activities.

463A Denitrification in riparian wetlands of the Seine River (France)
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The ecological functioning of the Seine estuary is strongly affected by the input of nitrogen, especially in the form of nitrate, which contributes to the eutrophication of the Seine Bight, (France). Modeling studies have shown that up to 40% of the nitrogen can be eliminated in riparian zones and between 15 à 20% in the water column, most likely due to denitrification. Elimination of nitrate by benthic denitrification in riparian zones or adjacent wetlands could significantly improve the water quality of the Seine estuary. The goal of this study was to investigate the potential for denitrification and the factors affecting in situ denitrification rates. To this end, we measured denitrification potentials using flow-through reactors in sediments collected from four sites along the Seine River during three different seasons. Sediment characteristics (organic C, C:N ratio, bioavailable carbon, chlorophyll, most probable number –MPN-) were determined and related to the potential denitrification rates. Denitrification rates showed a large spatial and seasonal variation and showed a significant correlation with sediment phytoplankton biomass, for example biodegradable carbon (expressed as chlorophyll concentrations). The addition of carbon, either in the form of simple organic molecules (acetate, lactate), reed or algae similarly increased the denitrification rates, indicating (i) a strong carbon limitation in these sediments and (ii) a lack of discrimination against the carbon source. The latter was confirmed through examination of the molecular structure of the sedimentary organic matter from 4 sites. Moreover, incubation of sediments for 2 months under denitrifying conditions did not induce any significant change in the chemical structure of the organic matter. In addition, the underlying bacterial community structure of the different sediments was analyzed by 16S rDNA and nosZ PCR-DGGE, trying to establish a relationship between the bacterial diversity, potential denitrification rates and environmental variables.
There is increasing evidence that global change can alter the structure of plant communities, with large impacts on the functioning of terrestrial ecosystems. However, still little is known about the impact of global change on soil microbial communities, and the interaction effects of different components of global changes on the soil microbiota remain poorly understood. In particular, the response of soil nitrite-oxidizers microorganisms that mediate the second step of nitrification, a key process of the nitrogen cycle, has never been investigated.

We examined the effects of four main global environmental changes on the activity, the abundance and the diversity of soil nitrite-oxidizers in an annual grassland ecosystem as part of the Jasper Ridge Global Change Experiment (CA, USA). This experiment includes four treatments - CO2, temperature, precipitation and nitrogen - with two levels per treatment (ambient and elevated, with elevated treatment based on prediction for the end of the century), and all of their factorial combinations. We measured potential nitrite oxidation, the abundance of soil Nitrobacter- and Nitrospira-like nitrite-oxidizers (using quantitative PCR targeting nxrA and 16S rRNA gene, respectively) and the diversity of soil Nitrobacter-like nitrite-oxidizers (using cloning-sequencing targeting the nxrA gene) in each treatment combination at the end of the 7th and 8th growing seasons under treatments. Furthermore, we analyzed to what extent changes in the activity of the soil nitrite-oxidizers result from changes in their abundance or diversity.

Simulated global environmental changes significantly altered the activity, as well as the abundance and the diversity of soil nitrite-oxidizers. Potential nitrite oxidation decreased with increased precipitation, but increased with elevated CO2 when combined with added nitrogen or precipitation. The abundance of soil Nitrobacter-like nitrite-oxidizers also decreased with increased precipitation and increased with elevated levels of CO2 and nitrogen. In contrast, the abundance of soil Nitrospira-like nitrite-oxidizers increased with enhanced precipitation, but decreased with elevated levels of CO2 and temperature. Finally, the structure of the soil Nitrobacter-like nitrite-oxidizers was significantly altered by the treatments. Consistently with results previously reported for agroecosystems, we found that changes in potential rates of nitrite oxidation in response to treatments was partly explained by changes in the abundance of soil Nitrobacter-like nitrite-oxidizers, but not by changes in the abundance of soil Nitrospira-like nitrite-oxidizers, suggesting that Nitrobacter-like nitrite-oxidizers were the main functional players of the soil nitrite-oxidizing microbial community. In addition, our results show that among the major populations of Nitrobacter-like nitrite-oxidizers, some were specific of particular treatments or treatment interactions and could be linked to activity levels.

Our study thus provides evidence that global change factors alter the enzyme activity, abundance and diversity of soil nitrite-oxidizers, with potential impacts for soil nitrogen cycling. We also demonstrate that the effects of elevated CO2, warming, increased precipitation, and enhanced N supply on the soil nitrite-oxidizing bacterial community are not simply additive, and therefore not predictable from single-factors experiments.

We determined the distribution and metabolism of planktonic prokaryotes inhabiting the deep-water masses of the South Atlantic Ocean across a transect from 52°69'S 49°55'W to 0°19'S 32°55'W, following the Antarctic Intermediate Water (AAIW). The abundance of microorganisms decreased with depth by one order of magnitude, while the microbial activity, assessed via leucine incorporation rates, decreased by 2 to 3 orders of magnitude from surface (50 m) to bathy- and abyssopelagic waters (down to 5000 m depth). In vivo respiration measurements of microbial organisms at 50m depth decreased from the Magallane Strait in the south towards the oligotrophic Southern Atlantic gyre and increased again towards the equatorial waters. Assuming that respiration is mostly associated to bacterial cells, the high respiration of microbes at the southern stations resulted in low bacterial growth.
efficiencies (BGE) for the communities inhabiting this area of the Atlantic. Prokaryotic autotrophic activity, as determined by dissolved inorganic carbon (DIC) fixation in the dark, was highest at the southern stations and decreased from the mesopelagic (~250m) to bathypelagic waters. To distinguish between the heterotrophic activity of Bacteria and Archaea, we measured leucine incorporation rates in combination with specific metabolic inhibitors for Bacteria and Archaea. Bacteria dominated the leucine incorporation in surface waters, contributing about 80% to total leucine uptake in these layers. This bacterial contribution declined to less than 50% below 2000m depth. Concurrently, the contribution of Archaea to total leucine incorporation increased from 20% in surface to ~60% in deep waters. The contribution of Bacteria and Archaea to the total leucine incorporation varied with latitude. The metabolic activity of specific bacterial groups was studied in detail using CARD-FISH combined with microautoradiography. Differences in the composition and heterotrophic activity of Bacteria were detectable between surface and deep waters but no clear latitudinal pattern was discernable. Alteromonas, SAR202 and SAR406 contributed more to the bacteria abundance and activity in deeper layers than in surface waters while the contribution to both abundance and activity declined with depth in the SAR11 cluster. Taken together, our results point to latitudinal and depth-related patterns in microbial metabolism in the Atlantic ocean, corresponding to differences in the distribution and contribution to the heterotrophic activity of Archaea and of specific bacterial groups. This latitudinal and depth-related variability in the autotrophic and heterotrophic metabolism suggests an influence of the inorganic nutrients as well as the quality organic matter on the composition and activity of the microbial communities inhabiting different areas of the South Atlantic Ocean.

465A Unexpected diversity of nitrite oxidoreductase genes (nxrB) in marine waters: indications for novel nitrite oxidizing microorganisms?
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Since the recent discovery of ammonia-oxidizing archaea (AOA), increasing evidence has accumulated that AOA are the main ammonia oxidizers in the ocean, where they can be extremely abundant and utilize minute concentrations of ammonia, which is oxidized to nitrite. However, nitrite rarely accumulates in the ocean but is either further oxidized to nitrate or serves as important link between aerobic and anaerobic N conversions in oxygen minimum zones (OMZ). Yet the organisms mediating nitrite oxidation in the ocean are poorly studied, and the abundance of known marine nitrite-oxidizing bacteria (NOB), i.e. Nitrospina, Nitrococcus, and Nitrospira, hardly correlates with nitrite oxidation rates.

The objective of this study was to identify putative NOB in the OMZ off the coast of Chile and Peru and to correlate their presence with chemical profiles in the water column. PCR-based approaches were developed to target 16S rRNA genes of the known NOB genera, and the gene encoding the beta subunit of nitrite oxidoreductase (nxrB). The only NOB-like 16S rRNA genes detected were affiliated with Nitrospina and revealed a large diversity within this group, but also a lack of correlation with the chemical profiles. In contrast, at least four distinct clusters of nxrB-like genes were observed, showing conspicuous depth distributions. The possibility that these genes indicate the presence of hitherto unknown NOB will be discussed.

466A Anaerobic bacterial arsenic reduction in a rare, hypersaline estuary ecosystem: the Laguna Madre (Texas, USA)
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The Laguna Madre is a rare, hyper-saline estuary. Migratory, rare, threatened and endangered species feed or nest in the Laguna Madre; however, the Laguna Madre has been impacted by anthropogenic chemicals, including arsenic. Laguna Madre sediments are a sink for arsenic whose presence in the Laguna Madre has been attributed to past pesticide usage, sewage discharge, and non-point sources. Microorganisms in the sediment may influence arsenic transformations such as the anaerobic reduction of arsenate (AsV) to arsenite (AsIII). Arsenate reduction releases soluble, toxic AsIII into the surrounding environment; thus, bacterial populations and communities mediating arsenate reduction are central to arsenic mobilizations and impact in the ecosystem. To characterize arsenate reduction in the Laguna Madre, arsenate-tolerant bacteria were first isolated. Sediment
samples were collected from 2 sites, designated Frank West and ABC, and inoculated onto solid media supplemented with 100 µM sodium arsenate. Densities of AsV-tolerant bacteria were estimated by viable plate counts. Plates were incubated anaerobically to culture potential AsV-reducing bacteria. Representative bacteria (n = 24) from AsV plates were assayed for carbon source utilization and enzyme activity using test strips. Bacteria were also challenged to grow on higher amounts of AsV, observed morphologically, tested for protein production, and identified by 16S rRNA gene sequencing. Results showed that the densities of AsV-tolerant bacteria were $7.7 \times 10^3$ and $9.7 \times 10^3$ colony forming units per gram (cfu g$^{-1}$) wet sediment for Frank West and ABC, respectively. Pure cultures could tolerate AsV concentrations as high as 60 mM; however, at higher AsV concentrations the growth of the cultures was slower, protein production was statistically lower, and the colony colors changed. Bacteria from site ABC displayed >88% phenotype similarity whereas bacteria from Frank West were less similar. Frank West organisms were able to utilize more than 10 carbon substrates and were better able to use certain substrates compared to ABC bacteria. Although representative bacteria showed phenotypic similarity, 16S rRNA gene sequencing revealed the presence of at least 13 genera. The most common genus identified was Bacillus. Bacteria are currently being tested for arsenate reduction rates. We conclude that sediments of the Laguna Madre are inhabited by a small but robust and diverse community of bacteria which may contribute to arsenic cycling in the ecosystem.

**467A Diversity of manganese-oxidizing bacteria isolated from Taklimakan Desert suggests diverse mechanisms for the interaction between bacteria and manganese**

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The Taklimakan desert is one of the most desiccate deserts in the world. The microbial community in this area has been poorly characterized so far. Previous studies suggest that manganese is an essential element for the desiccation resistant ability of the bacteria. Therefore, some of the bacteria in the desert could have manganese oxidation activity, and thus may play some roles in the geochemical redox cycle of manganese under aerobic condition. Here we isolated a total of 128 bacteria with strong Mn$^{2+}$ resistant activity from the sand of the desert, which are phylogenetically distant and belong to 21 genera. This microbial community is dominated by two bacterial phyla, the Firmicutes and Actinobacteria. These bacteria are able to efficiently oxidize Mn$^{2+}$ into insoluble manganese oxide with variable potency. Leucoberbelin blue assay together with the bacteria demonstrated that there are 11 different patterns for the localization of manganese oxides on the agar containing MnCl$_2$; it suggests diverse mechanisms for the bacteria to oxidize the Mn$^{2+}$. Manganese oxidation activity of the bacteria differs significantly in their dependences on Cu$^{2+}$. The biogenic MnOx chemicals produced by different bacteria differ in their structures. Moreover, we found that Mn$^{2+}$ induces differential chemical bond changes in the microbial cells. Mn$^{2+}$ also exerts diverse influences on energetic metabolism of these bacteria. Token together, we found a highly diverse microbial community with manganese oxidation ability existing in the Taklimakan desert. Our data provide insight into multiple mechanisms for the bacteria to oxidize manganese.

**468A Hitherto the nitrate transformations in Brazilian mangroves sediments**

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The mangrove ecosystem is a tropical coastal biome, located in the transition zone between land and sea, and is characterized by periodic flooding that gives these unique and specific conditions. These places have large amounts of organic matter that needs to be decomposed to supply nitrogen and other nutrients in the beginning of trophic chain. One of available form of nitrogen, the nitrate, plays an essential role in nature, acting in bacteria as a nitrogen source and also as an alternative electron acceptor. Some processes may compete for this nitrate, such as denitrification processes, reduction of nitrate to ammonium dissimilatory (DNRA) and anaerobic oxidation ammonium (anammox). In this study were screened three mangroves metagenomes for evidence of genes that encode enzymes
involved in the transformation of nitrate. In addition, in order to quantify these transformations we have used 15N-labelling techniques to compare the rates of the three processes in three mangroves systems. The mangroves sampled in this study are located in the city of Bertioga (Sao Paulo State, Brazil), and despite their near location in the same city, these mangroves are in different states of conservation: BrMgv01 and BrMgv02 were both affected by oil, but in distinct intensities (BrMgv02 the oil effects are still present, while in BrMgv01, it is not possible to observe effects of the oil spill); and BrMgv03 is affected just by the proximity to the city (subjected to waste water and other contaminants from city). The metagenomes used were generated by pyrosequencing (approximately 230,000 sequences each - 300bp average size) of environmental DNA, and the screened genes were involved in denitrification (nirS, nirK, noZ, norB, and also narG), anammox (hh, hao and hzo) and DNRA (nrfA). This approach selected a total 86 sequences related to the transformation of nitrate: being 61 related with denitrification, 19 with anammox, and 6 with DNRA. Sequences of genes hh, hao and hzo were mainly affiliated to Planctomycetes. In counterpart, the genes related with denitrification were phylogenetically distributed among different groups depending on the mangrove area analyzed (mostly affiliate within Gammaproteobacteria, Alphaproteobacteria and Betaproteobacteria); and DNRA-related sequences were affiliate only with Gammaproteobacteria. Determination of the three processes revealed the predominance for denitrification over the possible anammox transformation in all three mangrove areas studied. In addition, it was possible to observe that both processes, anammox and denitrification were suppressed in the oil affected area (BrMgv02). This area also shows highest amount of NOx consumed during incubations, indicating the low occurrence of DNRA activity. In summary, we have that metagenomic and functional approaches corroborates in affirm that denitrification process is most prevalent in these mangrove areas, while anammox and NOx consumption was also detected, as alternative metabolism for nitrate in mangrove sediments.

469A  Towards predictive understanding of nitrogen cycling in soils

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In anoxic environments, dissimilatory nitrate reduction to ammonium (DNRA) and nitrate reduction to N2O and N2 (denitrification) are the major nitrate/nitrite-consuming processes. DNRA oxidizes more C per mole of nitrate than denitrification and generates a cation (NH4+), which is retained in soils, indicating that these processes have profoundly different impacts on N retention and greenhouse gas (CO2, N2O) emissions. Microbes capable of DNRA or denitrification coexist but the regulatory controls for these competing processes are unknown, and their relative contributions cannot be predicted faithfully. This paucity of information limits the development of more accurate, predictive models of N-flux including the effects of N-retention on plant growth, as well as greenhouse gas emissions. To elucidate the environmental factors controlling nitrate/nitrite turnover in soils, a unique isolate (Shewanella loihica) that harbors both the DNRA and the denitrification pathways was studied. In addition, the impact of novel non-denitrifying N2O reducers on N2O flux was explored. The comparisons of gene-centric and genomic (who is there?) to the transcriptomic (who is how active?) datasets gathered from mesocosm experiments and agricultural soils provide system-level insights into the pathway controls and the functional redundancy within microbial communities controlling N-flux in soils.

Using Shewanella loihica harboring both complete denitrification and DNRA pathways, the effects of nitrate:nitrite ratios, quantity and type of C available, pH, temperature, and C:N ratios were explored. The results demonstrate that low nitrate:nitrite ratios, and the presence of external amino acids pronouncedly affected the choice of the predominant nitrate/nitrite reduction pathway. Another project goal is to better understand the diversity of nosZ genes involved in N2O reduction to N2. Genome analysis of the nitrite-to-NH4+-reducing bacterium Anaeromyxobacter dehalogenans strain 2CP-C revealed the presence of a complete, but atypical nosZ gene cluster. Physiological studies corroborated that this non-denitrifying bacterium uses N2O as a growth-supporting electron acceptor. Screening available bacterial genomes indicated that genera other than Anaeromyxobacter also possess atypical nosZ genes. The denitrifier- and atypical nosZ gene types share sequence similarities; however, the PCR primers used for environmental surveys of denitrifier nosZ genes failed to detect any of the atypical-nosZ genes. Newly designed PCR primers demonstrated the commonality of the atypical-type nosZ genes in a variety of soil ecosystems, suggesting that an important, yet unrecognized N2O sink exists. In parallel, novel primer sets targeting the nrfA gene associated with...
DNRA were developed. Isolation and sequencing efforts revealed an unexpected diversity of N₂O reducers and DNRA bacterial populations in soil ecosystems along with their associated genes. Apparently, current N-cycle models are missing possibly significant nitrite and N₂O sinks. To elucidate the relative abundance of genes implicated in nitrate, nitrite and N₂O reduction in soils, we have surveyed existing metagenome datasets. A bioinformatics pipeline was developed to identify and align short fragments of the target genes recovered in the available metagenomes. The preliminary analyses corroborated the commonality of atypical-type nosZ and nrfA genes in soil ecosystems, thus emphasizing that heretofore-unrecognized bacteria and genes contribute to N-cycle reactions.

470A  **NirK-possessing denitrifying bacteria, not fungi, produce N₂O in dairy manure compost**
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Composting, one of a major strategy for treating animal manure in Japan, produces greenhouse gas nitrous oxide (N₂O). Our previous studies using stable isotope approach showed that the N₂O emitted by compost surface and core show different isotopic signatures. While N₂O emitted from compost surface showed N₂O emission with known isotopic signature with bacterial denitrification process, the core samples amended with NO₂ showed unknown isotopic signature. Also, it is known that there are two groups of denitrifiers possessing nirS and nirK genes. However, it is not known that which group is important in N₂O production in compost. To narrow down the N₂O producer in the compost core, we used selective inhibition (SI) approach using the bactericide and fungicide.

Dairy manure compost (4 t in initial fresh weight) was used, and the piles were turned every 2 weeks. The pile surface and core were sampled at each turning event, incubated under mesophilic (30 °C for surface samples) and thermophilic (60 °C for core samples) conditions in the 100 mL vials, and headspace N₂O production after 24 hours was measured by GC-ECD. The bactericide (chloramphenicol) and the fungicide (cycloheximide) were used to partition the bacterial and fungal contribution on N₂O production. Diethyldithiocarbamate (DTC) was used to partition the nirS and nirK denitrifiers. The produced N₂O samples were used for the isotopomer analysis. Compost DNA was extracted, and nirS and nirK copy numbers were determined by qPCR.

For both surface and core samples, the N₂O production with site preference (SP) value around 0 (surface) and 15-20 (core) were strongly inhibited by chloramphenicol addition, indicates that bacteria is playing an important role for N₂O production in both samples. The cycloheximide addition did not inhibit or scarcely increased the N₂O production, which was observed in the presence of NO₂ amended core samples. After the incubation, NO₃ were detected from the NO₂ amended core samples. Also, the NH₄ content in the core samples decreased after the incubation, indicates co-denitrification utilizing NH₄ and NO₂ occurred in the core samples. While the nirS copy numbers were larger than that of nirK genes, the DTC addition inhibited both biotic and abiotic N₂O production almost completely, indicates that the nirK-denitrifiers are more important than the nirS-denitrifiers.

The microbes responsible for N₂O production in both compost surface and core with different SP values are bacteria in different phylogenetic groups. It was indicated that N₂O with unknown isotopic signature comes from the co-denitrification using NH₄ and NO₂.

470B  **Quantifying methane turnover in the deep sea: the Carlos Ribeiro Mud Volcano (Gulf of Cadiz) as a case study**
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The determination of the methane flux and consumption rates by microorganisms is of importance to constrain the methane budget and the carbon cycling in cold seep sediments. In deep-seated
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methane vents sites, such determination is complicated by the difference of gas solubility compared to ocean surface where gas concentrations are usually measured. In this study, we propose an original method to evaluate methane turnover in cold-seep sediments of the Carlos Ribeiro Mud Volcano (CRMV) located in the Gulf of Cadiz. Our approach combines ex-situ rates of methane oxidation and sulphate reduction, using radiotracer, together with methane profile resulting from reaction transport models (published in a companion paper Vanneste et al., Geochimica et cosmochimica ACTA 75: 4, 2011). Ex-situ rates were one order lower than methane and sulphate fluxes resulting from such models. This apparent inconsistency could be resolved by estimating in situ microbial activity rates based on modelled methane profiles. This suggested that the difference of methane solubility between in-situ vs. ex-situ conditions can lead to a large underestimation of the in-situ rates. A small fraction of the methane flux was oxidised in the bioirigrated shallow sediment subsurface, probably by an aerobic process, but the largest part was consumed by anaerobic oxidation of methane (AOM) coupled to sulphate reduction (SR). This activity involved all known types of anaerobic methanotrophs (ANME-1, -2 and -3). However, the AOM community in the MV crater centre was distinct from those at other seep sites as it was dominated by GoM-Arc1 Archaea and butane/propane oxidising sulphate reducing bacteria.

471A Denitrification in Bacillus vireti as a last resort to detoxify NO from DNRA
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Denitrification in Bacillus vireti as a last resort to detoxify NO from DNRA

Nitrate respiration allows microbes to grow under anoxic conditions and gain more energy than by fermentation. Denitrification is assumed to occur when nitrate is abundant. Under nitrate limited conditions, dissimilatory nitrate reduction to ammonium (DNRA) might prevail. This process reduces nitrate to nitrite and then further to ammonium. Nitrate is reduced by the same enzymes that can be found in denitrifying organisms, namely nitrate reductase (Nar) and/or periplasmic nitrate reductase (Nap). This reduction is always coupled to respiratory ATP synthesis. The reduction of nitrite to ammonium is done by cytochrome c nitrite reductase (NrfA). The only gaseous intermediate, NO, is usually enzyme bound and not released as in denitrification. Nonetheless has NO/N2O production been observed in DNRA. Some organisms, that carry N2O-reductase, would thus be able to reduce nitrate to N2 as a type of denitrification process. During the past decades much knowledge has been gained on the regulatory biology of denitrifiers, although most is based on studies of a few model strains of Gram negative bacteria. The role and regulation of DNRA performing organisms in natural environments is less well known.

In the present study, a large number of newly isolated Bacillus spp. was screened for end point products after growth on nitrate. Most strains produced only NO or N2O. One defined species which did produce N2, Bacillus vireti LMG 21834T, was studied in-depth in our robotic incubation system. This strain reduced all available nitrate to nitrite before it reduced the nitrite further. N2 production was negligible below initial nitrate concentrations of 5 mM, and ammonium was produced instead, presumably from DNRA. With increasing nitrate concentrations in the medium, about 50% of the nitrate was reduced to N2. At extreme nitrate concentrations higher than 40 mM, N2O became the prevailing end product of denitrification. Growth slowed down, once all available nitrate was reduced to nitrite.

Whole genome sequencing of B. vireti confirmed the presence of genes coding for Nap and NrfA, typical for DNRA, nosZ (coding for N2O-reductase) and a gene encoding Hmp, which could be the agent reducing NO to N2O. Genes coding for NO reductase (Nor) and the subunit NirC of nitrite reductase (Nir) were not found.

We hypothesize that B. vireti uses an alternative pathway for denitrification that is less energy yielding and mainly a way to detoxify NO. Even though NO is usually not released from NrfA during the reduction of nitrite to ammonium, this might occur under stress conditions like high nitrite concentrations. NO can potentially be reduced to N2O by Hmp and could then be further reduced to N2 by N2O-reductase. This would explain the slowdown of growth after reduction of nitrate to nitrite and the increase in N2O or N2 production with increasing concentration of initial nitrate in the medium.
Systematic gathering of phenotypic datasets for a range of denitrifying and other nitrate respiring organisms will provide a basis for the formulation and calibration of biogeochemical models aiming to predict responses of denitrifying microbial communities, and thereby greenhouse gas emission.

472A  Depicting biomass-degrading enzymes in mangrove sediments by metagenomics
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Mangroves are ecosystems located at the land-ocean interface in tropical and subtropical climates. Due to its proximity to the ocean, this biome selects for microbial inhabitants that are adapted to variations in salinity and those able to live under frequently low-oxygen conditions. This combination of environmental conditions makes the mangrove habitat organic matter-rich (approximately 10%), but nutrient-poor due to recalcitrant organic compounds degrading under the prevalent anoxic conditions. Thus, microorganisms play a role during this process in mangroves must be specially adapted to obtain nutrients from organic matter under such harsh conditions. In this study we aimed to assess the genes involved in the production of biomass-degrading enzymes (endoglucanase, endo-1,4-beta-xylanase, and chitinase) found at four mangrove sites (BrMgv01 to BrMgv04) using two complementary approaches: (1) in silico screening metagenomic datasets (~900,000 sequences) and (2) selecting fosmidial clones (based on functional assay) that encode for such enzymes (searching among 12,000 clones of approximately 40kb insertions each). The metagenomic analysis involved assembling sequences into contigs and then searching via BLASTX with the SWISSPROT (proteins) and PFAM databases. This approach revealed the following abundance of genes in decreasing order: chitinase-related (593 reads), xylanase-related (391 reads), and endoglucanase-related (325 reads). Shifts in frequencies of these sequences were observed at the first sampling site (BrMgv01), where we observed more endoglucanase-related genes. In contrast, there were much less xylanase-related genes. Concerning the fosmidial clones screening, we have so far found 2 clones with endoglucanase activity, 2 clone yielding xylanase, but no sequences revealed chitin-degrading ability. These contradictory observations (abundance vs. absence of chitinase-related clones) may indicate that functional enzymes in mangrove sediments require specific conditions, making this a very specialized environment when considering organic matter degradation. We have found that mangroves are hotspots that help us better understand possible transformations of organic matter and highlight the importance of exploring such environments from a biotechnology point of view.

473A  Impacts of climate change on N-cycling microorganisms in soil
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The effects of climate change on nitrogen turnover processes in soils are still not completely understood. Nitrification and denitrification are important drivers of N₂O emissions from soils, and emissions are increasing. The influence of elevated atmospheric CO₂ concentration, elevated temperature, changed precipitation patterns and drought on N₂O emissions and the abundance and activity of ammonia oxidizing and denitrifying microorganisms were investigated in five different field experiments in agricultural ecosystems (grasslands and arable fields). Elevated atmospheric CO₂ increased the growth and nutrient uptake of crop plants leading to decreased N₂O production rates in an arable soil, whereas ammonia oxidizer and denitrifier abundances were more affected by seasonal variations in soil moisture than by elevated CO₂. In a grassland ecosystem elevated CO₂ was found to increase N₂O emissions, but the CO₂ effect was restricted to sites with increased soil moisture. Elevated soil temperature doubled N₂O emissions in an arable field even though soil water content in the top soil layer was reduced as compared to soil under ambient temperature. Drought periods decreased the abundance of denitrifying bacteria in soil, but had only minor effects on potential denitrification rates. Data from the five experiments will be presented to offer insight into possible changes and feedbacks in the N-cycle in soils under future climate change scenarios.
474A  Heterotrophic growth of a marine bacterium from the SUP05/Arctic96BD-19 clade of gamma proteobacteria on organic carbon and thiosulfate
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Bacteria from the uncultured SUP05/Arctic96BD-19 clade of gamma proteobacterial sulfur oxidizers have the genetic potential to fix inorganic carbon and oxidize reduced sulfur in diverse marine environments including oxygen minimum zones, in the tissues of clams and mussels, and throughout the deep ocean (>200 m). Here we report on the heterotrophic growth of a novel culture from the SUP05/Arctic96BD-19 clade. Several cultures were obtained from surface waters in Puget Sound and from the deep chlorophyll maximum in the North Pacific gyre. A Puget Sound strain was selected for further study by reviving a culture from freezer stocks and verifying purity. The pure culture grows aerobically on natural seawater media and reaches higher cell densities when thiosulfate (1 µM) and organic carbon are added to the media, demonstrating the potential for sulfur oxidation to enhance organic carbon utilization. These data suggest that relatively low concentrations of reduced inorganic sulfur have the potential to increase organic carbon turnover in oxygenated seawater. We assigned the provisional taxonomic assignment “Candidatus: Thioglobus singularis”, alluding to the clade’s known role in sulfur oxidation and the isolate’s free-living lifestyle.

474B  Metagenomics-based mining of the microbial halogen cycle
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Modifications of bio-molecules via halogenation and dehalogenation reactions are a critical strand for the economy. These reactions are highly relevant to diverse industrial areas such as the manufacture of halogenated polymers, drug discovery, and bulk and fine chemicals. As they can be persistent pollutants, dehalogenation is also important in environmental issues, as corresponding activities and microorganisms can be effectively employed in bioremediation strategies. Current technologies, however, are not well developed for the identification and improvement of microorganisms capable of performing these specific reactions with advantages over traditional chemical processes. Furthermore, high-throughput screening is a particularly challenging task within an anaerobic environment favored by many dehalogenating microorganisms. These considerations make it difficult to switch to a biotechnological approach.

Therefore this project, in collaboration with MicroDish B.V., proposes to create a toolbox of complementary approaches: metagenomics, metabolic modeling, micro-fabricated culture-chip platform, and nano-reagents. This will enable the identification, study, and improvement of halogenating and dehalogenating microorganisms cultured under aerobic and anaerobic conditions, potentially covering the full width of microbially-mediated halogenation and dehalogenation conversions. In this there are three major aims: (1) To develop novel high-throughput approaches for the isolation, and functional characterization, of halogenating and dehalogenating organisms from different environments (2) to apply metagenomics and single-cell genomics to tap into the natural diversity of halogenating/dehalogenating consortia, and (3) to apply molecular breeding approaches to generate novel biocatalysts and biosensors targeting a wide range of halo-organic pollutants.

For the sources of halogenating and dehalogenating microorganisms, we will focus on environments containing halo-organic compounds due to pollution or natural production. These include sites polluted with halogenated aliphatic and aromatic compounds. In addition, we will select samples from natural sources of halo-organics, including marine sponges, algae, and marine sediment, for which the spectrum of produced halogenated compounds is known.

Thus far a PCR-based screening method using degenerate primers was developed to screen a wide range of metagenomic samples for the presence of halogenases and dehalogenases. Samples were from natural, man-made, and enriched environments. Results indicate a number of potential environments of interest for halogenases and dehalogenases. These samples will be further examined by pyro-sequencing to elucidate the sequences.

Using a novel culturing platform, the MicroDish Cultivation Chip, an activity screening for halogenase and dehalogenase activity in environmental samples was developed. This screening strategy takes
advantage of the high-density culturing format of the chip, and its ability to change conditions without disrupting colonies, together with colorimetric and fluorescent activity detection reagents to culture and isolate microorganisms with halogenase and dehalogenase activity. The outcomes of these culturing and activity screenings will be discussed in the poster.

475A  Microbial methanization in organic-rich shales
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There is increasing evidence for the microbial transformation of deeply buried organic matter into methane in the deep subsurface. Microorganisms involved in methanogenesis have been previously identified in coal beds and oil reservoirs. However, little is known about the indigenous communities of shales, e.g. organic-rich laminated sedimentary rocks. Our study aims at 1) quantifying and identifying the presence of methanogens in organic-rich shales, 2) assessing the influence of rock chemistry on microbial diversity and activity, 3) determining the molecules preferentially biodegraded in order to evaluate the methanogenic potential of immature, organic-rich shales.

A core was drilled in the Lower Toarcian shales of the Paris Basin, from 7 to 30 meters depth. Samples were collected every ten centimeters on the core for geochemical and petrological characterizations and microcosm setup. Methane production was monitored by Gas Chromatography over a 1-year period. Microbial populations were identified and enumerated by 454 sequencing and qPCR of the bacterial and archaeal 16S rRNA genes, or the mcrA gene for methanogens. The geochemical characteristics of the shale were determined by Rock-Eval 6 pyrolysis. To evaluate the preferentially degraded molecules, the rock was gradually extracted with organic solvents to remove different organic fractions, e.g. saturated and aromatics hydrocarbons, resins and asphaltenes. The extracted rock samples were incubated and monitored as above.

Our results show that methanogens are present in extremely low numbers in situ, below the detection threshold, but can be detected and quantified after cultivation. We show a perfect correlation between the presence of methanogens in microcosm and methane production. Sequence analyses revealed a low diversity among Archaea, which are composed of only methanogens of the order Methanosarcinales. Surprisingly, we found that the mcrA gene allowed the numeration of only 10% of the methanogens. Bacteria exhibited a larger diversity. Three major phyla were identified: Firmicutes, Bacteroidetes and Proteobacteria, including several genera of fermentative (syntrophic), acetogenic, and sulfate-reducing bacteria. Thus, methanogenesis in shales follows the accepted methanogenic scenario describing a cooperation of syntrophs and methanogens for the mineralization of organic matter.

Microbial communities, and consequently methane production, were unevenly distributed in the shale with depth. No clear correlation could be established between microbial activity and rock geochemistry. Our results show that methane yields increase with the progressive withdrawal of the soluble fractions of the organic matter from the rock matrix. This is contradictory with the proposed model for coal and petroleum degradation, which assumes a microbial preference for smaller molecules during the degradation sequence.

These results raise several questions with regards to methanogenesis in organic-rich rocks. The production of methane from high molecular weight organics by the consortia implies that methanization in situ might extend to heavier organic fractions than supposed to date. However, it remains to determine whether these heavy fractions are readily accessible to methanogens in situ.

476A  Land use changes in tropical peatlands affect microbial and metabolic processes
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Peatlands are unique yet critical natural resources that form 70% of global wetlands, hold 10% of global freshwater resources and they remarkably sequester 1/3rd of world's soil carbon. More than 50% (22 million hectares) of the tropical peatlands are in Indonesia. These peatland forests hold around 42 Gigatons of soil carbon and refuge huge biodiversity (1.4 –1.8 million species). These peatlands are under threat from human pressures mainly through drainage and deforestation accompanied with forest fires to bring them in use for agriculture and rehabilitation purposes. This, in turn, is leading to oxidation of peat biomass by microbial processes to produce 0.8 Gigatons CO2 per year, constituting nearly 3-8% of global emissions due to fossil fuels. Our 11 study transects are located near Jambi, Sumatra island covering 48 sq km and differing in peat depth, land cover, water table, states of degradation and management systems. The team has been gathering data on land-use pattern from remote sensing and on-site measurements for gas emissions, microbial diversity and geochemical parameters at various depths during dry and wet seasons for past few years.

Here we report, that water table and change in land use pattern play an important role in shaping microbial community structure. Based on nutrient analysis, nitrates were highly associated with the palm oil plantation sites whereas mixed crop plantations have high association with salinity followed by ammonium. Metabolite profiling revealed that metabolic signatures were better indicators for stratification of peatland sites when compared to microbial rDNA fingerprints. Based on clone data, majority of the bacteria belonged to Firmicutes bacillales, Alphaproteobacteria, Actinobacteria and Gammaproteobacteria. Wood degradation studies were done to indicate peat biomass decomposition potential of microbes. Significant wood decomposition (recorded as weight loss) above (27-94%) and below (8-18%) the water table was observed in less than 4 months.

Deep sequencing metagenomics was performed from samples from three land use pattern and a total assembly of 26% aligned reads was found based on 4.2 million reads. Comparison of orthologous gene categories among the three land use types has provided valuable insights into the effects of land use type on microbial communities. In future, we hope to validate the microbial functions associated to peatland microbial ecology based on metagenomic analysis.

477A Seasonal changes in the community structure of ammonia-oxidizing prokaryotes in oligotrophic groundwater from limestone aquifers

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Microbial nitrogen transformation processes in aquifers play an important role for the suitability of groundwater as a drinking water resource. Here, nitrification is a key step. While numerous studies focused on terrestrial or marine systems, only little is known about the microbial communities mediating ammonia oxidation, the first and rate limiting step of nitrification, in groundwater ecosystems. In this study, we investigated the abundance, community composition and activity of ammonia-oxidizing archaea (AOA) and bacteria (AOA) in groundwater obtained from a shallow and a deep karstic limestone aquifer situated in the Hainich region (Thuringia/Germany). The goals of this study were (i) to assess nitrification potential of the groundwater, (ii) to compare the community composition and abundance of AOA and AOB between the shallow (12 m) and the deep aquifer (48 and 88 m), and (iii) to follow temporal changes in the abundance and community composition of AOA and AOB over a one-year period.

Aquifers differed strongly in oxygen saturation and concentrations of nitrate, which were higher in the deep (44.3 %; 117 µmol liter\(^{-1}\)) compared to the shallow aquifer (15.3 %; 3.8 µmol liter\(^{-1}\)) while concentrations of ammonium usually remained below 10 µmol liter\(^{-1}\) in both aquifers. Nitrification potential determined in microcosm incubations amended with \(^{15}\)N-ammonium yielded rates of 0.4 ± 0.15 nmol [NO\(_x\)] liter\(^{-1}\) hour\(^{-1}\) in the deep aquifer. Community composition and abundance of AOA and AOB was analyzed using a combined DGGE/cloning approach and quantitative PCR targeting the ammonia-monoxygenase subunit A (amoA) gene as a molecular marker. The amoA gene copy numbers (8.9 \times 10\(^{4}\) to 2.6 \times 10\(^{7}\) liter\(^{-1}\) for AOA and 2.9 \times 10\(^{6}\) to 4.5 \times 10\(^{8}\) liter\(^{-1}\) for AOB) were 1-2 orders of magnitude lower for both AOA and AOB in the shallow compared to the deep aquifer, suggesting that oxygen availability was a key factor influencing the distribution of ammonia oxidizers in the aquifer system. AOA-amoA/AOB-amoA gene ratios varied by three orders of magnitude over the
course of one year, indicating predominance of AOA in early summer and predominance of AOB during the winter months. Similarly, DGGE based analysis showed a variation in community composition of AOA and AOB over time but without changes in the major phylotypes present. Both DGGE and sequence analysis revealed clear differences in the community composition of AOA and AOB between the two aquifers. Interestingly, AOA communities were more diverse than AOB communities, which were dominated by one phylotype closely related to Nitrosomonas ureae.

Our results demonstrate that ammonia oxidizing prokaryotes are present and active in oligotrophic groundwater and their abundance and community composition is influenced by key geochemical parameters such as oxygen availability. Factors underlying the strong seasonal fluctuations of AOA/AOB ratios and abundances in the aquifer system remain to be investigated.

477B Ecophysiology of a large planktonic marine Alteromonas that consumes an unexpectedly high fraction of dissolved organic carbon
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The oceanic pool of dissolved organic carbon is equal in magnitude to carbon dioxide in the earth’s atmosphere. In the upper ocean, this material is predominantly regulated by heterotrophic activity of Bacteria and Archaea. It has traditionally been suggested that phylogenetic diversity is required to achieve significant reductions in dissolved organic carbon, as it provides a wider community repertoire of ectoenzymes and transporters necessary for hydrolysis of macromolecules and subsequent uptake of hydrolysis products. We tested the hypothesis that a single bacterial isolate is capable of metabolizing the same proportion of ambient dissolved organic carbon as native microbial assemblages. After screening multiple isolates, Alteromonas sp. ALT199 was chosen as the model for this study based on its ability to grow rapidly on ambient dissolved organic carbon in unamended, filter-sterilized seawater. The ecophysiology of the isolate was described by assessing its growth performance when faced with competition and grazing pressures. Mesocosm experiments compared the carbon consumption of: 1) native free-living prokaryotes; 2) ALT199 in isolation; and 3) free-living prokaryotes plus ALT199 inoculated at 1% of the total population. Three seasonal regimes with initial ambient total organic carbon concentrations spanning 70-89 μM were sampled and used as prokaryotic inoculum and ambient dissolved organic carbon for drawdown experiments. Growth of Alteromonas sp. ALT199 in isolation resulted in equal carbon drawdown compared with the native microbial assemblage, regardless of initial dissolved carbon concentration. Furthermore, the addition of ALT199 to the native microbial assemblage resulted in a two-fold increase in the percent of carbon consumed relative to either treatment individually. Bacterial growth efficiency of ALT199 was 32% lower than the native prokaryotic community, yet fluorescence in situ hybridization revealed that ALT199 outcompeted its competitors and rapidly became dominant (>65% of total). Surprisingly, ALT199 did not alter its size to compete with small prokaryotes, to escape grazing pressure, or in response to diminished nutrient availability over time. This study suggests that: 1) Broad phylogenetic diversity is not required for significant consumption of ambient dissolved organic carbon; and 2) Large sized, relatively sparse morphotypes within the prokaryotic community have the potential to provide a disproportionately large contribution to carbon flux, both through total bacterial carbon demand and as a direct conduit to protozoan grazers. These findings are significant since relatively minor variations in bacterial growth efficiency or increased metabolic potential to consume dissolved organic carbon can results in large-scale changes global carbon flux and atmospheric carbon dioxide concentrations.

478A Abundance of functional groups of microorganisms as predictors for potential biogeochemical process rates
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Nitrification and denitrification are central processes in nitrogen cycling. They are important to local and global soil functioning, since they lead to mobile forms of nitrogen for plant uptake, nitrogen leaching and losses of the greenhouse gas nitrous oxide to the atmosphere. In order to thoroughly understand the biogeochemistry of nitrogen cycling it is crucial to unravel the major environmental predictors for nitrification and denitrification. The major objective of this study was to evaluate which environmental factors were important in explaining measured patterns in potential nitrification and
denitrification rates across five major plant community types in interior Alaska. More specifically, we tested (1) whether potential process rates could be predicted solely from soil physical and chemical characteristics or (2) if the abundance of functional genes could be an additional explanatory variable. Surface soils were sampled along an elevation-driven hydrologic gradient at the Bonanza Creek Long-Term Ecological Research station that represents five plant communities typical of interior Alaska. The plant communities included a black spruce stand, a deciduous stand, a tussock grassland, an emergent fen, and a rich fen. We examined the chemical composition of the surface organic moss and soil, measured gross N-mineralization, potential rates of nitrification and denitrification, and quantified abundances of several functional groups of microorganisms from soil cores collected in mid summer. We used quantitative PCR to assess the functional gene abundances of bacterial and archaeal ammonia oxidizers (amoA genes) and denitrifiers (nirS, nirK and nosZ genes). We used path analysis to test possible causal relationships between measured chemical composition, potential process rates and functional gene abundances. In path analysis, experimentally supported theory is used to formulate a conceptual model of the causal and non-causal relationships between the measured explanatory variables and their dependent variables, in this case potential nitrification and denitrification processes. Across the soils from the five different plant communities, we found based on path analyses that abundances of functional genes were the most important explanatory variables and the major controlling factors for the potential biogeochemical rates measured in this study. Bacterial amoA genes and nosZ genes were the variables that best explained the variation in potential nitrification and denitrification rates, respectively. Chemical factors such as ammonium, nitrate and organic matter content were less correlated to potential process rates, but influenced the process rates indirectly through the functional gene abundances. This study shows, that quantitative DNA-based information of functional groups can provide important information about the patterns and rates of nitrogen cycling processes in the environment. This quantitative relationship between microbial populations and biogeochemical process rates is highly applicable and could be established as a sensitive indicator to track large-scale and long-term changes in patterns of biogeochemical cycling.

479A  Impact of silver nanoparticles on nitrification in estuarine sediments
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Silver nanoparticles are well known antimicrobial agents and are incorporated into several consumer products including textiles, food packaging, personal care products and medical devices. Consequently, nanosilver is released into wastewaters and enters environmental systems, however little is known about their fate, behaviour or potential risks there. In aquatic ecosystems, nanosilver has the potential to disrupt in situ microbial communities and their processes. Nitrification is crucial to the global cycling of nitrogen and is an important process for the removal of ammonia from wastewaters. Ammonia-oxidation is the key, rate-limiting step of nitrification and is mediated by ammonia-oxidising bacteria and archaea. The aims of this study were to physically and chemically characterise nanosilver, measure their toxicity to ammonia-oxidisers, and investigate their effect on nitrification in estuarine sediments downstream of a wastewater treatment plant.

Size characterisation revealed that the nanosilver were monodisperse (average diameter 35 nm ± 0.2) in milliQ water but underwent aggregation (60 nm ± 0.5) in estuarine water (27 %salinity). At concentrations of 0.5 mg L⁻¹, nanosilver had a toxic effect on ammonia-oxidising bacteria, causing significant reductions in the nitrification rate of Nitrosomonas europaea (from 34 to 3 μM NH₄ day⁻¹) and Nitrosospira multiformis (from 50 to 3 μM NH₄ day⁻¹). At 5 mg L⁻¹ nanosilver caused a significant reduction in the nitrification rate of Nitrosococcus oceani (from 26 to 18 μM NH₄ day⁻¹).

In freshwater and mid-salinity sediments, 0.5 mg L⁻¹ nanosilver had no effect on nitrification potential, however higher concentrations (50 mg L⁻¹) caused up to a three-fold reduction in nitrification potential in both sediment types after one day exposure. After fourteen days exposure, nitrification potentials remained significantly reduced in freshwater sediments (12 μM NH₄ day⁻¹), but recovered in mid-salinity sediments (35 μM NH₄ day⁻¹). PCR-DGGE analysis revealed distinct shifts in ammonia-oxidising bacterial and archaeal communities in freshwater and mid-salinity sediments when exposed to high concentrations of nanosilver (50 mg L⁻¹), resulting in the disappearance of some DGGE bands after 7 days.
Our results suggest that 0.5 mg L\(^{-1}\) nanosilver has a detrimental impact on nitrification by ammonia-oxidising bacteria, and 50 mg L\(^{-1}\) can cause shifts in ammonia-oxidiser community composition in sediments. The global nanotechnology industry is rapidly expanding, leading to an exponential increase in the use of nanosilver in consumer products. Nanosilver in European sediments is said to be increasing at a rate of 952 ng kg\(^{-1}\) y\(^{-1}\) (Gottschalk et al., 2009), thus the risk of nanosilver pollution is fast rising. It is therefore vital that we better understand the fate and behaviour of nanosilver in aquatic environments and their effect on microbial communities involved in global nutrient cycles and other important processes, so that we can prevent or minimise their disruption in the future.

Motivated by the question of microbial processes in Precambrian banded iron formations (BIFs) (3.8-1.8 Ga), we investigated the cell-mineral aggregates produced by modern anoxygenic Fe(II)-oxidizing phototrophic and nitrate-reducing, Fe(II)-oxidizing bacteria. These cell-mineral aggregates can be described as networks of Fe(III) minerals and cells with a constant stoichiometric excess of Fe(III) compared to the autotrophically fixed carbon in the biogenic precipitate (Posth et al., 2010). A large percentage of biogenic minerals is utilized during early diagenesis; these minerals are highly reactive (Sobolev and Roden, 2001) and easily cycled by Fe(III)-reducing bacteria (Straub et al., 1998).

Yet, little is known about the storage or transformation of organic matter associated with Fe(III) minerals. As Fe-OC aggregates in old, deep sediment would undergo transformation processes driven by temperature and pressure diagenesis, understanding how they are altered over deep time is of fundamental importance to the cycling of Fe and C, but also to our interpretation of these elements in the rock record. For this reason, we have begun investigation of the thermal and barometric alteration of precursor Fe(III) minerals associated with organic carbon. A simplified system was tested in which mixtures of chemically-synthesized ferrihydrite (biogenic Fe mineral proxy) and glucose (biomass proxy) were sealed into gold capsules and treated in autoclaves at 170°C and 1.2 kbar (diagenetic conditions estimated for the Transvaal BIF, S. Africa). Iron speciation and mineralogical analysis show the conversion of ferrihydrite (Fe\(_{x}\)(OH)\(_{3}\)) into hematite (Fe\(_{2}\)O\(_{3}\)), magnetite (Fe\(_{3}\)Fe\(_{2}\)O\(_{4}\)), and siderite (Fe\(_{3}\)CO\(_{3}\)). When silica-coated ferrihydrite was used, hematite and siderite, but no magnetite was microscopically detected. Interestingly, this demonstrates the production of minerals found in BIFs today by the joint deposition and burial of microbial biomass and primary Fe(III) minerals followed by increased temperature and pressure during diagenesis. Whether this describes the long term fate of organic carbon in ancient iron sediments still needs to be fully explored. Current experiments focus on 1) the potential pathway of organic carbon produced by anoxygenic S- and Fe-oxidizing phototrophs from the point of carbon fixation, through precipitation to sedimentation and burial as tracked by \(\delta^{13}C\) and 2) the potential preservation of biomarkers and microbial fossils during pressure-/temperature-induced diagenesis. The experimental system presented herein offers a means to explore C and Fe cycling over geological time and bridge the gap between modern biogeochemical analogues and the rock record.

Responses of redox potential, nitrogen loss and soil microbial communities to frequent changes in aerobic and anaerobic conditions on a flooded soil

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Human activities interfere with the natural flooding regime of floodplains bordering rivers. As a result of a dyke construction, the frequency of river flooding is often reduced. Today, the restoration of biodiversity and its functions in human-disturbed wetlands is an important ecological topic. Flood pulses are one of the major disturbances in a floodplain system. They strongly influence the pathways of nitrogen cycling by including changes in physical, chemical and microbial properties of the floodplain soils. We carried out a study in the Seine River floodplain (France). Our objectives were to better understand the effect of alternate aerobic and anaerobic conditions on structure of soil microbial degrader communities and an ecological function (denitrification). The effect of hydroperiod on redox potential, loss of added and native nitrogen and soil microbial communities was investigated under flooded soils mesocosms during 7 and 14 days. The evolution of redox potential, N\textsubscript{2}O evolved and soil microbial communities throughout the experiment were determined by redox micro-electrode, gas chromatography with ECD and quantitative real-time PCR method, respectively. We investigated the evolution of total, denitrifying and sulfate-reducing microorganisms by targeting bacterial 16S rRNA, nitrous oxide (nosZ) and dissimilatory sulfite reductase dsrAB genes respectively. Alternate flooding and draining water increased nutrient removal efficiency compared to the continuous flow and control systems. The redox potential of the soil decreased more slowly under alternate aerobic and anaerobic conditions than under continuous anaerobic conditions. The denitrification process increase. This high rate of denitrification was due to alternate aerobic-anaerobic period. The size and composition of the soil microbial community convincingly distinguish between soils. Our results show that the structure of total microbial community was not affected by the frequent changes in aerobic and anaerobic conditions.

481A  A quantitative model of bacterial particle and polymer degradation in aquatic systems

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Particulate and polymeric organic matter are the main organic substrates found in drainage and wastewater, and in natural aquatic systems. Their abundance in the environment makes them important substrates in microbial carbon cycling. Utilisation of these substrates require synthesis and excretion of extracellular depolymerising enzymes, enzymatic depolymerisation, polymeric intermediates formation and subsequent uptake and growth. In order to understand the combined effect of reaction and transport processes in open ecosystems, for example structured systems like lake and oceanic sediments and biofilms, these processes needs to be separately defined and formulated into an overall model. Several conceptual models have been proposed attempting to describe cycling of particulate and polymeric organic matter in aquatic systems. In order to avoid excessive model complexity, most models omit the role and dynamics of extracellular enzymes (like the activated sludge models used in biological wastewater treatment) and/or leave out degradation particle and polymer intermediates.

In this work a conceptual model describing particle and polymer turnover is presented including extracellular enzyme dynamics and intermediates formed during the depolymerisation process. Furthermore, the model is mathematically formulated including stoichiometry and kinetics of all sub processes. The mathematical model is formulated as a series of differential equations based on elucidated stoichiometries and established kinetics of individual bacterial growth and decay processes, and extracellular enzyme activities. The model is implemented in the aquatic modelling tool AQUASIM, a model sensitivity analysis is performed to identify key parameters for the overall particle and polymer dynamics, and a short evaluation of model complexity and potential simplifications is given.

The proposed model reflects interactive degradation and transport phenomena typically found in gradient systems. In addition, the model may also contribute significantly to our understanding of ecological growth interactions, like competition for organic substrate in natural systems and process selective pressures in environmental biotechnological process systems. Finally, it is our intension to apply this model for experimental design of a series of tests for identification of identified key model parameters.
**482A  Biomethylation of mercury by earthworms and its influence on ingested bacteria**

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Methylmercury (methyl-Hg) is very toxic for most organisms. Earthworms with their activities in nutrient cycling and soil formation are important members of the soil fauna. They are known to accumulate mercury and methyl-Hg. Methylation of inorganic Hg has been mainly observed under anoxic conditions but the knowledge of this process in earthworms are far from complete.

Here in a laboratory experiment with our model species Lumbricus terrestris the potential of earthworms to methylate inorganic Hg was investigated. We hypothesized that the anaerobic, nutrient-rich condition in the earthworm digestive tract leads to methylation of Hg by their gut inhabiting bacteria.

Earthworms were either grown in sterile and untreated soils, or soils treated with inorganic HgCl₂ or organic CH₃HgCl. Control experiments were also conducted abiotically and with bacteria collected from earthworm surfaces. After 30 days of incubation, the soil and earthworm samples were analyzed for their total and methyl-Hg concentrations. Additionally, the effects of Hg and methyl-Hg on the bacterial community structures and compositions in soils and earthworms were studied by T-RFLP profiling of the 16S rRNA genes and cloning approach.

Tissue concentrations of methyl-Hg in earthworms which grew in Hg-treated soils were about seven times higher than in earthworms from non Hg treated soils. Concentrations of methyl-Hg in the soils and earthworm casts remained constant over time. Therefore we assume that Hg is mainly methylated in the earthworms. The different types of Hg treatments have significantly influenced the bacterial community structure and composition. Soil bacterial communities were mostly affected by inorganic Hg, whereas bacterial communities in earthworms were affected by the methyl-Hg treatment. About 11% of bacterial OTUs in earthworms were significantly affected in soils treated with Hg and almost 20% of OTUs were significantly influenced in soils treated with methyl-Hg compared to non Hg treated soils. OTUs affiliated with Firmicutes were sensitive to Hg and methyl-Hg whereas OTUs most similar to Betaproteobacteria were tolerant to the Hg treatments. Interestingly, sulfate-reducing bacteria were found in earthworms but not in soils.

We conclude that earthworms are able to methylate inorganic Hg. Earthworms which grew in Hg treated soils contained seven times higher methyl-Hg than earthworms from non Hg treated soils. We also found evidence that sulfate-reducing bacteria in earthworms could be potentially involved in the methylation of Hg in earthworms.

**483A  Denitrification in natural freshwater systems – analysis of local and continental determinants**

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Natural freshwater systems (for example lakes, streams) have an important role in reducing the load of anthropogenic nitrogen from land to vulnerable coastal ecosystems. This takes place through sedimentation of organic N compounds and through emissions of N₂ gas due to microbially mediated denitrification and anaerobic ammonium oxidation (anammox). Although quantitation of N₂ gas producing processes have been done in many freshwater systems, studies on the structure and diversity of N₂ gas producing microbial communities in natural freshwater systems are especially scarce. Consequently, there is only very limited knowledge about the factors affecting N₂ gas production in these systems. This information, however, would be very important when the response of the processes to changes in ecosystem, such as increase of nitrogen loading, is predicted. The aim of this study was to assess the variations in the activity and the structure of N₂ gas producing communities in sediments of freshwater systems focusing especially on lakes. A general view of the factors affecting N₂ gas production in natural freshwater systems was gained by combining the data of our own measurements and from previous publications.
The study was done in two scales: in the local as well as in the continental scale. In the local analysis the activity and the structure of N\(_2\) gas producing communities of sediments was studied for the spatial and temporal variations in 5 boreal lakes and one stream. N\(_2\) gas production rates were analysed using the isotope pairing technique (IPT) and microbial communities using molecular microbiological methods (for example DGGE of nirK and 454-pyrosequencing of nirS, nirK and nosZ, and 16S rRNA gene amplicons). In the continental scale analyses previously published data on denitrification rates (measured using IPT) and environmental factors was collected from studies of 9 lakes located in Northern, Central and Southern Europe. The data was analysed using correlation, regression and multivariate analyses.

Denitrification was the only significant N\(_2\) gas producing process in the study lakes. Denitrification rates varied temporally and spatially both within and between lakes ranging from 50 to 1400 µmol N m\(^{-2}\) d\(^{-1}\) and from 0 to 12900 µmol N m\(^{-2}\) d\(^{-1}\) in the local and continental scales, respectively. N\(_2\) gas production of the stream could not be reliably assessed, but the lower denitrification potentials than those observed in lakes indicate that the stream had a very limited capacity to remove N. The molecular microbiological analyses are still underway, but preliminary results indicate that the community structure of nirS, nirK and nosZ-carrying communities did not affect process rates. Thus, environmental factors seem to be the dominant controllers of N\(_2\) gas production in the study lakes. Nitrate concentration in the water above the sediment was the dominant controlling factor and affected positively denitrification rates in both geographical scales. After accounting for the effect of nitrate concentration, the influence of oxygen concentration and temperature on denitrification rates was also shown.

483B  Methane microbial ecology in hydroelectric reservoirs: methanotrophic activity in water samples and of a bacterial strain from Tucuruí reservoir, Pará, Brazil
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Among the greenhouse effect gases (GHG) emitted from hydroelectric reservoirs, methane receives special attention, since it is the gas whose production is most stimulated by the flooding of the reservoir area and the hydroelectric plant operation regime. This increase in methane production is due to microbial methanogenic activity in the sediments and the final emission balance is also influenced by the methane consumption by methanotrophic bacteria along the water column. Methanotrophic bacteria are aerobic proteobacteria known for their ability of using methane as the only source of carbon. In this work, methanotrophic activity was assessed in microcosms inoculated with water from two different depths in the reservoir (7 and 20m) and with a methanotrophic culture isolated from the same area. Reservoir water microcosms were set in triplicate in 100mL flasks, containing 54mL NMS mineral medium closed under atmosphere of methane: air (10:90) and inoculated immediately after sampling with 10%v/v water samples. A methanotrophic strain, isolated in previous incubations from samples of the same area was assessed under the same conditions. Methane consumption was measured by gas chromatography with flame ionisation detector and cellular growth determined by counts of DAPI stained cells under fluorescence microscopy. Determination of methanotrophic activity was calculated from linear regression of the higher inclination section of the curve.

Methane was completely consumed after 200 to 220 hours in flasks inoculated with water samples and after 265 hours in the pure culture incubation. Increase in cell concentrations observed by DAPI counts ranging from 10\(^5\) to 10\(^6\) cells mL\(^{-1}\) and no significant methane loss in control flasks confirmed that methane consumption was due to bacterial activity. The microcosms inoculated with water sampled at 7m depth showed a lag phase of about 120h before presenting a methanotrophic activity of 1.13±0.07mmols CH\(_4\) h\(^{-1}\), while microcosms incubated with water sampled at 20m depth showed a constant consumption of 0.74±0.28mmols CH\(_4\) h\(^{-1}\) along all incubation period. Differences in culture behaviour may be related to variations in composition and activity of methanotrophic communities following combined gradients of methane and oxygen along the reservoir water column. The pure culture incubation revealed a methane consumption activity of 0.69mmols CH\(_4\) h\(^{-1}\) after a lag phase of 70 hours. The culture was isolated from a water sample collected near the surface of the reservoir, and is composed by gram negative cocobacilli. Sequencing of the 16S rRNA for identification of the strain is being carried out. Results obtained confirmed the presence of active methanotrophic bacteria.
along the water column of the Tucuruí reservoir, indicating that the biological methane consumption influences the methane emission balance in the area.

484A  Impact of nitrification on the Nitrogen Cycle in the Elbe estuary  
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The supply of bioavailable nitrogen is crucial to primary production in the world's oceans. Especially in estuaries, the N-cycle has a particular significance and acts as a nutrient filter for costal waters. Nitrogen sources for primary production and microorganisms are dissolved inorganic nitrogen (DIN, ammonium, nitrite and nitrate) but also dissolved organic nitrogen (DON) or particulate nitrogen. Reactions in this N-cycle are mainly driven and controlled by microorganisms. Nitrification is therefore one key reaction, divided in two sub processes the ammonia and nitrite oxidation which are carry out by ammonia oxidizing bacteria (AOB) and archaea (AOA), respectively and nitrite oxidizing bacteria.

Due to fertilisation, the Elbe estuary is loaded with nitrogen. In the past, it acted as nitrogen sink, but today the situation changed and the estuary turned from a sink to a source of nitrogen, while the absolute concentrations are decreasing. Especially the maximum turbidity zone in the Elbe estuary is an active turnover site of reactive nitrogen, but its role in filtering nutrients is yet to be fully understood and quantified.

During three cruises in August 2011, March and May 2012, we measured nutrient concentration, d15NNO3 and nitrification rates along a transect from the river mouth through the maximum turbidity zone to the port of Hamburg. We compare these nitrification rates with isotope fractionation factors of the relevant microorganisms.

Reactive nitrogen in the river is mainly available as nitrate, and both the isotope signals and high nitrification rates suggest combined nitrification/denitrification in the maximum turbidity zone.

Our results show that the river Elbe is a significant source of reactive nitrogen for the coastal North Sea. The responsible process appears to be nitrification, actively recycling nutrients and adding to the already elevated anthropogenic nitrate loads in the river. This is underscored by tremendously high turnover rates in the MTZ, which obviously actively regulate nitrification and overall N-Cycling in the Elbe estuary.

485A  The contributions of fungi to the biogeochemical cycling of Mn in metal-rich environments  
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It is widely accepted that microbial activities contribute substantially to the biogeochemical cycling of manganese, an essential element for life, in diverse environments. For example, microorganisms promote Mn redox reactions, either Mn(II) oxidation or Mn(IV) reduction, through direct enzymatic or indirect pathways. Experimental studies show that the rates of microbiologically-catalyzed Mn(II) oxidation are up to several orders of magnitude faster relative to abiotic catalysis, thus it is hypothesized that microorganisms are the primary drivers of Mn(III/IV) oxide formation in surface environments. Most of the research on the geomicrobiology of Mn(II) oxidation to date has been devoted to understanding the pathways, mechanisms, and products of Mn(II)-oxidizing bacteria. However, Mn(II)-oxidizing fungi have been also recovered from a wide variety of marine, freshwater, and terrestrial surface environments. It is becoming increasingly apparent that fungi contribute to the biogeochemical cycling of Mn, potentially as much as, or even more than, bacteria.

We sought to identify and characterize the Mn(II)-oxidizing microbial communities living in metal-rich environments, because these organisms could potentially contribute to the remediation of contaminated environments through the precipitation of insoluble Mn(III/IV) oxide minerals. We utilized culture-based approaches to obtain Mn(II)-oxidizing microorganisms from several acid mine drainage treatment systems in Pennsylvania as well as from a freshwater pond, Ashumet Pond, in
Massachusetts. All of these sites had greatly elevated concentrations of dissolved metals, particularly soluble Mn(II) compounds. Additionally, we performed a metagenomic pyrosequencing survey of these environments to examine the distribution of the Mn(II)-oxidizing microorganisms relative to the total microbial community.

Through the culture-enrichment surveys, we isolated a diversity of both Mn(II)-oxidizing bacteria and fungi from all field sites. The majority of isolates obtained from Ashumet Pond were bacteria, representing 7 different phylotypes. Although fewer Mn(II)-oxidizing fungi were isolated from this environment, the isolates represented 5 different Ascomycete species. In contrast, 9 different fungal Ascomycete species accounted for 90% of the total isolates obtained from the constructed passive remediation systems in Pennsylvania, and only 4 bacterial phylotypes demonstrated the ability to oxidize Mn(II) in culture. Interestingly, many of the fungal species from these biographically distinct locations were phylogenetically related. The culture-based results suggest that, at least in certain environments, Mn(II)-oxidizing fungi may be contributing significantly to the biogeochemical cycling of Mn. The metagenomics surveys of these field sites indicated that the Mn-oxidizing phylotypes were potentially a small fraction of the total microbial community, however more quantitative measurements of the key microorganisms as well as the relative activity of these organisms is warranted.

486A Isolation and characterization of novel thermophilic nitrifying Bacillus sp. from compost
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Composting is a general treatment for recycling animal wastes as plant fertilizer. During the treatment, however, a lot of nitrogen is lost. Most nitrogen losses result from ammonia emission. Ammonia emission produces an unpleasant odor and is a source of irritation for neighbors of large-scale composting operations. Nitrifying bacteria oxidize ammonia and reduce the smell of ammonia, making them ideal potential organisms for animal waste bioremediation. Thermophilic and heterotrophically nitrifying bacteria isolated from compost are needed for biological deodorization of ammonia in compost. The authors designed a new C-1 medium containing organic matter to approximate the composition of the complex composting environment (Shimaya and Hashimoto 2008). The C-1 medium has several advantages over the conventional medium used for culturing thermophilic and heterotrophically nitrifying bacteria from compost. Thermophilic nitrifying bacteria in compost must be cultured at temperatures of more than 50 °C, which are generated by the composting process (Shimaya et al. 2003). The C-1 medium supports reasonable growth of thermophilic nitrifying bacteria at 50 °C, and allows quantification of thermophilic nitrifying bacteria during the composting process. A thermophilic nitrifying bacterium, strain T3, was isolated from compost made of animal wastes by using the C-1 medium. Strain T3 was classified into the genus Bacillus, close to Bacillus halodurans, but identified as a novel species. To evaluate the effect of adding strain T3 on ammonia emission during the process of composting animal wastes, laboratory scale composting was done. Ammonia emission was lower when strain T3 was added than in the control material to which strain T3 was not added. Thermophilic nitrifying bacteria in the strain T3-containing material increased from 6.24 (log value) to 7.55 (log value) on average during the tests. These results suggested the possibility of reducing ammonia emission from composting of animal wastes by adding strain T3.

487A Heterotrophic nitrate assimilating bacteria throughout the Atlantic ocean: new perspectives on the global nitrogen cycle
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Recently, major advances in our understanding of the oceanic nitrogen cycle have been accomplished with the identification of new pathways. One of the less studied topics in the deep ocean is the fate of nitrate and its role in deep water heterotrophic bacterial metabolism. Nitrate can serve as electron acceptor or as nitrogen source for both autotrophic and heterotrophic organisms. We hypothesized that nitrate might be a significant source of nitrogen for heterotrophic bacteria in deep waters and oligotrophic oceanic regions, where dissolved organic matter is highly refractory and low in nitrogen content, whereas nitrate is readily available. To test this hypothesis, the gene encoding the assimilatory nitrate reductase (nasA) of heterotrophic bacteria was characterized and quantified from
surface to bathy- and abyssopelagic waters in different oceanographic regions in the Atlantic characterized by different trophic conditions. The nitrate assimilating bacterial community was phylogenetically variable within and between different regions and depths, including members related to Bacteroidetes, Alphaproteobacteria and Gammaproteobacteria. The nitrate assimilating communities were significantly different in the different depth layers with the exception of the polar North Atlantic, where the deep water formation might be responsible for a more homogeneously distributed community over different depth layers. The relative abundance of thenasA gene was higher in deeper waters than in surface waters. Taken together our results point to an utilization of nitrate as an N source in deep water heterotrophic Bacteria.

488A  Nitrite Respiration in a Groundwater Contaminant Plume, Cape Cod, Massachusetts, USA
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Nitrite is a key intermediate in all known nitrogen dissimilatory processes (NDPs). Its reactivity and thermodynamic potential allow it to be oxidized or reduced; with both reactions occurring concurrently in certain situations. The pulse of N-respiration in an environment can be assessed by quantifying the fate and kinetics of nitrite turnover. Contamination with dissolved inorganic nitrogen (DIN) is an increasingly common situation in groundwater, where denitrification is usually viewed as the predominant NDP. However, other NDPs could be important to the overall fate and transport of subsurface DIN. We examined NDPs in a wastewater-contaminated groundwater plume on Cape Cod with the focus on nitrite turnover. Natural gradient tracer tests using 15N-NO2 in the anoxic zone of the plume resulted in production of 29N2 and 30N2 from anammox and denitrification, and nitrate from either nitrification or nitrite oxidation by anammox. Rates of nitrite oxidation were somewhat higher than nitrite reduction, though both were low (<1 μmol L^{-1} day^{-1}), but consistent between multiple, downgradient wells. Biomass, collected from water samples through filtration and incubated in an anoxic chamber, produced both nitrate and trace amounts of oxygen when exposed to high concentrations of nitrite. Quantitative PCR of hydrazine synthase (hzsA) and nitrous oxide reductase (nosZ) revealed that anammox bacteria were much more abundant than nitrous oxide-respiring, denitrifying bacteria in the aquifer. In addition, sequence analyses of 16SrRNA and methane monoxygenase genes revealed the presence of nitrite-dependent, anaerobic, methane-oxidizing (n-damo) bacteria, which are capable of producing molecular oxygen from nitrite. If molecular oxygen was released by n-damo bacteria, it may have supported nitrite oxidation by aerobic nitrite oxidizers during the tracer tests. Thus, this study demonstrated that while the apparent predominant NDP in the anoxic portion of the plume is net N2 production, intermediate N pathways can be complex and can involve both aerobic and anaerobic nitrite-oxidizing processes. Anammox and n-damo-related bacteria appear to be active and in close proximity to each other in this unconfined aquifer.

489A  Coral mucus enhances prokaryotic productivity in surrounding seawater
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Corals release mucus to their surrounding environment. Released coral mucus is rich in inorganic nutrients such as ammonium and phosphate, and organic carbon and nitrogen compared to the surrounding seawater. Coral mucus containing nutrients and organic matters is utilized by organisms in the surrounding environments as well as coral holobionts. Additionally, coral mucus contains much higher abundance of prokaryotes than those in the surrounding seawater. Coral mucus plays an important role as a carrier of energy and nutrients, and is responsible for biogeochemical processes in the reef systems. Some of the previous studies investigated the variations of organic carbon concentration and/or bacterial abundance when corals released the mucus to the surrounding seawater. However, it is still unclear which prokaryotic fraction, originating from the seawater or the mucus, is activated by the released coral mucus. We measured prokaryotic productions affected by coral mucus with tritium labeled thymidine (Tdr) and leucine (Leu) methods. Seawater and coral samples were collected in October 2011 and April 2012. Corals used in this study were Acropora sp., Favia sp., Favites sp. and Goniastrea sp. The corals were exposed to air for 10 min to obtain pure coral mucus. Seawater or 0.22-µm-filtered seawater were amended with the obtained mucus at a ratio of 1:300 in an sterilized polycarbonate bottle. Prokaryotic productions in seawater were 54.2 ± 11.3 (mean ± standard deviation) pmol TdR L^{-1} h^{-1} and 848 ± 314 pmol Leu L^{-1} h^{-1}. Prokaryotic productions
in seawater amended with the mucus (MuS) of Acropora sp., Favites sp. or Goniastrea sp. were significantly higher than those in seawater (87.5 ± 20.2 pmol TdR L⁻¹ h⁻¹ and 1236 ± 229 pmol Leu L⁻¹ h⁻¹, \( p < 0.05 \)); meanwhile, prokaryotic productions in filtered seawater (MuFS) were significantly lower than those in seawater (3.88 ± 4.18 pmol TdR L⁻¹ h⁻¹ and 40.4 ± 26.2 pmol Leu L⁻¹ h⁻¹, \( p < 0.01 \)). Although a similar trend of the lower prokaryotic production in MuFS of Favia sp. was observed (8.20 ± 11.43 pmol TdR L⁻¹ h⁻¹ and 15.2 ± 9.8 pmol Leu L⁻¹ h⁻¹, \( p < 0.01 \)), prokaryotic productions of the MuS was not always higher than those in seawater (54.2 ± 31.5 pmol TdR L⁻¹ h⁻¹ and 905 ± 221 pmol Leu L⁻¹ h⁻¹, \( p > 0.1 \)). The Leu:TdR molar ratio in MuFS (7.9 ± 7.4) was relatively lower than that in seawater (14.0 ± 3.2). These results suggest that the enhancement of the prokaryotic productivity in the surrounding seawater by coral mucus was due to the increased number of seawater prokaryotes, and not by the prokaryotes from the mucus. However, the effects varied among coral species. Mucus release plays an important role in biogeochemical processes in reef systems by enhancement of prokaryotic productivity in the surrounding seawater.

489B  High affinity sulfate reduction kinetics in marine sediment
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Sulfate-reducing bacteria (SRB) play a key role in the terminal mineralization of organic matter in marine sediments. The kinetics of bacterial sulfate reduction and the degree to which low sulfate concentrations can limit the reduction rates has previously only been inferred from observations at high sulfate concentrations. Such studies have shown apparent half-saturation concentrations (\( K_m \)) values ranging from 0.1 to 2 mM and thus indicated that lower sulfate concentrations may limit sulfate reduction rates in marine environments where the sulfate concentration is below this range. In contrast an abundant and diverse community of SRB is typically present in such environments suggesting a potential for SRB to sustain sulfate-reducing activity at low sulfate concentrations.

We aimed to resolve whether high affinity sulfate reduction occur in marine sediments. Sulfate reduction kinetics was investigated in a marine coastal sediment and in the marine SRB Desulfobacterium autotrophicum using two independent experimental approaches: (i) "Initial velocity experiments" where sulfate reduction rates was determined from \( ^{35} \text{SO}_4^{2-} \) tracer turnover under different initial concentrations of sulfate in individual subsamples. (ii) "Progress curve experiments" where sulfate was progressively depleted during time course incubations. To achieve sufficient analytical precision at diminishing sulfate concentrations the latter incubations were also spiked with \( ^{35} \text{SO}_4^{2-} \) tracer. The radiotracer experiments were supported with a highly sensitive ion chromatographic technique for sulfate with a lower detection limit of 150 nM.

We find that sulfate reduction proceeds at low sulfate concentrations in both the sediment and in \( D. \) autotrophicum. The apparent kinetics of sulfate reduction in the sediment could be explained by two dominating affinities for sulfate: a low affinity with a mean apparent \( K_m \) of 430 \( \mu \text{M} \) \( \text{SO}_4^{2-} \) and a high affinity with a mean \( K_m \) of 2.6 \( \mu \text{M} \) \( \text{SO}_4^{2-} \). For \( D. \) autotrophicum a similar shift from low to high affinity sulfate reduction occurred with a mean \( K_m \) value of 9 \( \mu \text{M} \) when sulfate concentrations decreased below 500 \( \mu \text{M} \).

The presence of high affinity sulfate reduction in marine sediment and in a marine sulfate-reducing bacterium shows that some SRB indeed have the potential to effectively utilize low sulfate concentrations. High affinity potential for sulfate reduction is therefore not a distinct physiologically adaptation for SRB living under sulfate limitations. The presence of both low and high affinity sulfate reduction in \( D. \) autotrophicum also imply that the two distinct apparent affinities for sulfate reduction observed in the marine sediment might not necessarily be due to the activity of different sulfate-reducing populations. SRB seem to have the ability to change their physiologically properties and thereby the affinity for sulfate reduction in response to changing sulfate concentrations.
Ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) co-occupy every agricultural soil that has been examined to date. However, little is known about what influences the relative contributions of AOA and AOB to soil nitrification. As a consequence, there is considerable interest in finding a selective inhibitor to distinguish between AOA and AOB activity in situ. With this in mind, we compared the growth response of the AOB, Nitrosomonas europaea, and the AOA Nitrosopumilus maritimus, to C2 – C10 alkynes. Whereas C2 – C10 alkynes completely prevent growth by N. europaea, N. maritimus growth was not affected by C8 – C10 alkynes over a similar concentration range. Previously, we developed an assay that discriminates between AOA and AOB contributions to ammonia oxidation in soil slurries by inactivating ammonia monooxygenase (amo) with acetylene (C2), and then following the recovery of the nitrification potential (RNP) in the presence and absence of a mixture of bacterial protein synthesis inhibitors to which archaeal protein synthesis is insensitive (Taylor et al., 2010). Two soils were identified in which the relative contributions of AOA and AOB differed. The RNP of a permanent pasture soil was mostly attributed to AOA (90 ±20 % of RNP was antibiotic resistant), while RNP of a recently N fertilized, wheat cropped soil was mostly attributed to AOB (10 ±10 % of RNP was antibiotic resistant). In slurry assays, octyne (C8) at concentrations > 10 µM completely inhibited nitrification potentials in both soils. However, at µM differential sensitivity of the two soils was measured. Approximately 80% of the nitrification potential in AOA dominated pasture soil was insensitive to octyne concentrations ≤10 µM, whereas octyne concentrations between 10 and 0.1 µM completely inhibited the nitrification potential of AOB dominated wheat cropped soil. In addition, RNP in pasture soil recovered equally well in the presence of either octyne or antibiotics. Further studies are in progress to critically evaluate if octyne or other long chain alkynes might be a simple and inexpensive inhibitor to discriminate between AOA and AOB contributions to nitrification activity in soils.

The effect of pH on soil microbial community structure and function: results from a manipulated pH experiment

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It is well known that microbial communities and associated functionality differ greatly across natural soil pH gradients. However, performing comparative experiments to examine the explicit relationship between microbial diversity and functioning with soil pH in natural systems is problematic due to the presence of multiple confounding parameters.

In this study we sought to examine the role of pH in shaping soil microbial community structure and associated soil processes over a manipulated pH gradient. Soil was sampled from the Craibstone site (Scotland) where soil has been artificially maintained at pH 4.5 and pH 7 for over 40 years. Soils were incubated with glucose and wheat stem to represent a labile and recalcitrant C resource respectively. Throughout the incubation total respiration and substrate specific respiration were measured to examine soil carbon processing. Microbial communities were monitored using phospholipid fatty acid (PLFA) and terminal restriction fragment length polymorphism (T-RFLP) analyses.

Microbial communities were found to be strongly affected by soil pH, exhibiting variability in community structure comparable to that which exists across natural environmental gradients. Similarly, PLFA analysis revealed microbial community structure differences between the low and high pH soils. Significant differences in soil C processing were also observed, but contrary to expectations, basal respiration and substrate decomposition were consistently higher in the low pH soil. The increase in soil respiration processes in low pH soils was unexpected as these soils had lower microbial diversity and were dominated by assumed oligotrophs. However, indicative of higher biomass, total PLFA abundance was greatest in the low pH soils showing that increased functioning can occur in soils of reduced microbial diversity if biomass is higher. We therefore demonstrate the importance of soil pH as a driver of taxonomic diversity and functioning in the absence of confounding factors. However we cannot generalize that the direction and magnitude of the functional response is in any way determined by either diversity or soil pH.
Wood decomposition is strongly influenced by physical and chemical properties of woody species as well as by environmental conditions. These factors form the basis for most predictive forest wood decay models but do not account for the variation in wood decay rates at smaller temporal and spatial scales. A large fraction of mass (and carbon) loss of woody materials actually takes place during the first decade, for which the predictions of current models have the lowest accuracy. This hampers the extrapolation of short-term, site-based measurements to larger temporal and spatial scales, consequently reducing the reliability of carbon sequestration estimates. The reason for this mismatch could be the underlying assumption of current models that community dynamics of decomposing organisms is not important for predicting decay rates, although it is generally acknowledged that the identity and type of rot fungus largely affects the process and rate of wood degradation.

LOG-LIFE is a new long-term 'common-garden' experiment to disentangle the effects of species' wood traits and site-related environmental drivers on wood decomposition dynamics and its associated diversity of microbial and invertebrate communities. LOG-LIFE features two contrasting forest sites in the Netherlands, each hosting a similar set of coarse logs and branches of 10 tree species. In one of these sites, an add-on experiment was set up in which many replicate logs of two tree species (Larix kaempferi and Quercus rubra L.) were placed on the forest floor in order to investigate how dynamics of wood-rot fungal communities contribute to the prediction of local variation in wood decay rates. For each tree species, 16 subplots host 9 logs. The logs, 30 cm long and about 20 cm in diameter, were sawn from 8 individual trees for each species. Each year, we will take one wood block of each subplot to the lab for analyses. Since we incubate the wood on the same forest soil, we attempt to minimize the between-subplot variability in abiotic conditions. In this way, and in combination with 16 replicates per time point, we hope we can demonstrate the range of decomposer-related variation in wood-decay. The experiment will run for approx. 10 years and will be of interest to microbiologists, entomologists, biogeochemists and ecologists alike, all of whom we encourage to help us get an integrated understanding of decomposer–wood interactions.

Identification of acetate-oxidizing bacteria in Aarhus Bay surface sediment by RNA-stable isotope probing in anoxic slurries and intact sediment cores

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In coastal marine sediments the sequence of electron-accepting processes consuming $O_2$, $NO_3^-$, Mn(IV), Fe(III), and $SO_4^{2-}$ is found on a small spatial scale near the surface and acetate is considered a major substrate for the microorganisms that convey the anaerobic terminal electron-accepting processes. We wanted to investigate the acetate-oxidizing bacteria of Aarhus Bay surface sediment (0-0.5 cm depth) in slurry incubations (which are often conducted for stable isotope probing (SIP)-experiments) and additionally in intact sediments cores. Acetate-oxidizing bacteria were identified by 16S rRNA-SIP with $^{13}C$-acetate addition to intact cores and anoxic slurries over 4-5 days of incubation. In parallel, electron-accepting pathways were monitored for porewater and solid phase constituents and sulfate reduction rates measured by $^{35}S$-tracer incubations. In the intact core incubations oxygen, nitrate, manganese, iron, and sulfate were all available and likely all used as electron acceptors by the microbial community in the surface sediment, whereas only microbial iron and sulfate reduction co-occurred in the slurries. Sulfate reduction was stimulated by acetate addition in both slurry and intact core incubations but mixing of the sediment in the slurries also seemed to stimulate the process. The acetate addition resulted in partial accumulation of acetate in the slurries whereas in the core incubations acetate was completely consumed within 19-24 hours. Members of the Oceanospirillaceae were identified as acetate oxidizers in both types of incubations by RNA-SIP. Additionally, bacteria related to Colwellia and Arcobacter oxidized $^{13}C$-acetate in the intact core while members of the Desulfuromonadales and Acidithiobacillaceae did so in the slurry incubation. Members of the two latter groups are capable of iron reduction and likely oxidized acetate coupled to iron reduction in the acetate-amended slurries where iron reduction accounted for 42% of total carbon oxidation. Interestingly, RNA of known sulfate-reducing groups was not labeled with $^{13}C$ although sulfate reduction was stimulated by the addition of acetate. The electron acceptors used by the
acetate-oxidizing bacteria related to Oceanospirillaceae, Colwellia, and Arcobacter in the intact sediment cores, cannot be assigned because several potential electron acceptors were available.

The comparison of slurry and intact cores incubations showed that (i) rates and zonation of microbial processes in the intact sediment cores resembled the natural conditions more closely, whereas in the slurry incubations mixing of the sediment and imposing of anoxia interrupted the tight coupling of the microbial processes and altered the electron donor and acceptor usage. (ii) The electron acceptor availability selected the acetate-oxidizing bacteria in intact core versus slurry incubations. The lack of $^{13}$C-labeling of sulfate reducers in the slurry incubations additionally indicated that RNA of organisms using electron-accepting pathways with lower energy yield is more slowly labeled with $^{13}$C. This might indicate a lower detection limit in SIP studies for the electron-accepting pathways with lower energy yield which does not necessarily correlate with the importance of the process.

494A Extracellular enzyme activity assays as a tool to investigate priming in freshwater biofilms
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Priming is the enhanced mineralization of recalcitrant organic matter (ROM) upon addition of labile organic matter (LOM). This effect is well described for terrestrial ecosystems but has not yet been addressed in aquatic ecosystems. In streams, microbial biofilms play a key role in carbon cycling and due to the matrix-enclosed lifestyle of bacteria and algae, biofilms might be hotspots for priming. While algal primary production delivers LOM, bacterial cells produce extracellular enzymes which are involved in the degradation of ROM. The activity of extracellular enzymes can be regulated by OM availability and hence might be a useful tool to detect priming. In this study we experimentally investigated the extent to which priming contributes to ROM degradation in stream biofilms. We used bioreactors to grow heterotrophic biofilms, which were adapted to a ROM-rich environment. Priming was induced by the addition of glucose, a combination of glucose and inorganic nutrients and an algal extract. We measured the activity of glucosidase, xylosidase, cellobiohydrolase, leucine-aminopeptidase, endopeptidase, esterase, phosphatase and phenol oxidase in the biofilms. PLS (projections of latent structures) modeling showed a clear separation of the treatments with priming and the controls for all enzyme activities. More specifically, the activity of endopeptidase, phenoloxidase and esterase were enhanced in the presence of LOM. This is first evidence for priming in freshwater biofilms.

494B Sulfur isotope fractionation in various habitats around mud volcanoes and brine pools in the Gulf of Mexico
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Brine pools, brine basins, and mud volcanoes are common features in the Gulf of Mexico. Their origin is warm, hypersaline, anoxic fluids, which flow from the deep subsurface upwards through seafloor sediments. Transfer of brines, crude oil, and gas from deep reservoirs to the overlying sediment and water column drives accumulation of dense chemoautotrophic communities along the seafloor. Depending on fluid composition and flow rates, microbial communities show a high metabolic variability between the seafloor brines.

Dissimilatory sulfate reduction and anaerobic oxidation of methane are dominate processes in the carbon cycle of these environments. During microbial transformations of sulfur compounds, sulfur isotopes are fractionated and in return, their isotopic composition provides information of the microbial pathways by which the compounds were formed. During microbial sulfate reduction, sulfide is typically depleted in light isotopes relative to sulfate. The degree of fractionation during microbial sulfate reduction depends on factors like organic substrate supply/substrate limitation and temperature, which drive changes in sulfate reduction rates.

Natural sulfate and sulfide isotopes ratios ($^{34}$S/$^{32}$S) from various habitats around mud volcanoes and brine lakes showed remarkable variability between sites. At Garden Banks 425, a actively venting mud volcano, $^{34}$S/$^{32}$S of the reduced sulfur compounds where high (up to +15‰) whereas the sulfate
isotope ratios were in the range $+21$ to $+32\%$, both sulfur compounds showed little down core variability. In contrast, at brine pool GC 246, the isotope ratio of the reduced sulfur species increased with depth from $-10$ to $+4\%$. Sulfate isotope ratios also increased down core with values reaching $48\%$ at the deepest sampling depth of 15cm. These isotopic values reflect variability in environmental biogeochemical signatures and sulfate reduction and anaerobic methane oxidation rates, as well as possibly microbial community composition.

495A  **Microbial ecology of Anammox organisms: do organics drive Anammox?**  
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Anaerobic Ammonium Oxidation (Anammox) has been shown to be an important player in the global nitrogen cycle. However, despite this high-level of interest, there is still much that we are yet to fully understand about these unique and interesting bacteria. This project aims to acquire a better understanding of the diversity and distribution of Anammox in the Eastern Tropical North Pacific (ETNP) Oxygen Minimum Zone (OMZ) and over a salinity gradient along an estuary in the UK. Furthermore it aims to look for the possibility of new, potentially heterotrophic routes to the Anammox process in these environments. To achieve these aims we utilised an in-depth, high-throughput methodology (including the use of 454 pyrosequencing) to gain a more comprehensive picture of the biogeography of these organisms. We also used Stable Isotope Probing (SIP) to follow the fate of organic-N substrates through these environments and into cell biomass. Validation of Anammox specific 16S rRNA primers has been conducted allowing us to be able to preferentially distinguish diversity across environmental gradients. Analysis of $^{12}$C and $^{13}$C labelled DNA shows not only an enrichment of Anammox bacteria with the addition of organic compounds but a shift in Anammox diversity between Candidatus Scalindua spp. in the inorganic $^{12}$C DNA fractions and Candidatus Kuenenia spp. and Candidatus Brocadia spp. in the organic $^{13}$C DNA fractions. This data suggests that not only are Anammox bacteria capable of utilising organic-N compounds but that certain genera within the Anammox clade have a greater capacity for these substrates. These findings have the potential to add a new and previously unseen slant on the anaerobic nitrogen cycle in these environments and provide a new understanding of the Anammox process.

496A  **New insights into the Movile Cave food web: bacteria using methylated amines as a carbon, energy and nitrogen source**  
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Movile Cave is an unusual underground ecosystem which was discovered in Mangalia, Romania less than thirty years ago. Prior to its discovery, the cave was completely sealed off from the outside for over 5 million years. Nonetheless, the cave hosts an astonishingly rich and diverse population of microorganisms, fungi and cave-adapted invertebrates. In the absence of light and with no organic carbon entering from above, life in Movile Cave is sustained exclusively by non-phototrophic microbial carbon fixation. Microorganisms that oxidise methane or reduced sulfur compounds are among the major primary producers, giving rise to extensive microbial mats on the limestone walls of the cave and on the surface of the thermal, sulfidic waters, kept afloat by rising methane bubbles. In addition to methane-oxidising bacteria, a range of non-methanotrophic methylotrophs have been detected in Movile Cave, including *Methylotenera mobilis*. These bacteria use one-carbon compounds such as methanol and methylated amines as their sole source of carbon and energy. Produced during decomposition of organic matter, methylated amines are likely to be major degradation products in the cave. In addition to being methylotrophic substrates, methylated amines are also a nitrogen source for a wide range of non-methylotrophic bacteria. The aim of the present study was to reveal the role of methylated amines as a carbon, energy and nitrogen source for bacteria in Movile Cave, using a combination of DNA stable isotope probing, cultivation and functional gene probing. To identify active methylamine utilisers, a time course enrichment was set up with cave water and $^{13}$C-labelled methylamine. Analysis of labelled DNA by denaturing gradient gel electrophoresis and high-throughput sequencing revealed a shift in the bacterial community over time. *Methylotenera mobilis* completely dominated heavy fractions after several days of incubation, while at later time points it had been replaced by a more diverse methylotrophic community including species of *Methylobacterium* and *Methylphilus*. Interestingly, results from DNA stable isotope probing and cultivation also uncovered a novel methylotroph, identified as a member of the genus *Catellibacterium*, as one of the dominant methylamine-utilising bacteria in Movile Cave. Presently available functional gene markers for
methylamine-utilising bacteria target the *mauA* gene, encoding methylamine dehydrogenase, which converts methylamine to formaldehyde in the direct methylamine oxidation pathway. However, these primers only detect some groups of methylotrophic methylamine utilisers. We designed primer sets targeting *gmaS*, the gene encoding gamma-glutamylmethylamide synthetase, the first enzyme of the recently characterised indirect methylamine oxidation pathway involving the intermediates gamma-glutamylmethylamide and N-methylglutamate. The indirect pathway was prevalent amongst both methylotrophic and non-methylotrophic Movile Cave isolates, as revealed by polymerase chain reaction based screening. The *mauA* gene was only detected in a number of methylotrophic isolates, generally in addition to *gmaS*. The new *gmaS* primers are currently being tested on environmental samples and have great potential as a biomarker for identifying a wide range of methylamine-utilising bacteria not detected by current primer sets that target only methylotrophs possessing the *mauA* gene.

**497A** Quantitative detection of nitric oxide reductase (*norB*) gene and nitrous oxide reductase (*nosZ*) gene in soils of different land-use types

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Nitric oxide (NO) and nitrous oxide (N₂O) are involved in global warming and the exhaustion of the ozone layer. Since soil is the main source of atmospheric nitrous oxide, it is important to know the abundance of bacteria capable for the conversion of NO to N₂O as well as the bacteria able to reduce N₂O. In this study, real-time PCR was used to evaluate two denitrifying genes - nitric reductase gene (*norB*) and nitrous reductase gene (*nosZ*)- in soils under different land use types. Soils were collected from four sites from an experimental field design of EMBRAPA-CPAO, Dourados, southwest of Brazil: (a) conventional tillage (CT); (b) crop/livestock integration (CLI); (c) pasture (PA) and (d) forest (F). Total DNA was extracted from soil samples and used as template for SYBR green quantitative real-time PCR assays. For amplification of *norB* gene, it was employed the primer pair cnorBFF/cnorBRR and for *nosZ* gene, nosZ2F/nosZ2R. Statistical analysis was performed using the general linear model SAS. Significance was accepted at a level of probability (P) of <0.05. The abundance of *norB* gene ranged from 10⁵ to 10⁶ copies g⁻¹ of dry soil. The quantification of *norB* gene in the pasture soil was significantly higher than in others types of land use. Differences between crop/livestock integration and forest were not significant and conventional tillage presented the lowest quantification. The abundance of *nosZ* gene was found at 10⁶ copies g⁻¹ of dry soil and was significantly higher in crop/livestock integration soil. No significant differences were found between conventional tillage, pasture and forest. Comparing the abundance of the two genes investigated in this study, *nosZ* gene was high for all land use types. These results suggest that in all types of land use evaluated in this study, there are more bacteria converting nitrous oxide to atmospheric nitrogen (N₂) therefore there is a low emission of N₂O, an important greenhouse gas which has a global warming potential 296 times higher than that of CO₂.

**498A** Vanadium addition to contaminated sediments results in vanadium reduction, decreases diversity and selects for specific taxa

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Vanadium (V) is both a widespread environmental contaminant from fossil fuel combustion and a poorly understood, but commercially important, metal. Although several vanadium reducers have been isolated from the environment, none have been isolated on vanadium from highly contaminated sites or have been implicated in bioremediation. Injection of organic carbon directly into an aquifer to biostimulate contaminated sediments in Rifle, CO has resulted in the removal of up to 68 µM (96%) of contaminant vanadium from groundwater for an extended period of time (months to years). This process occurs concurrent with iron reduction. In order to better evaluate the microbial role in vanadium removal, we used in-well flow-through columns to allow the simultaneous addition of acetate (a carbon source) and additional (5 mM) vanadate - V(V) - to sediments along with the influx of natural groundwater. In these experiments we measured vanadium loss by colorimetric methods, enumerated numbers of V(V) reducers present before and after and stimulation using the most probable number (MPN) method, isolated vanadium reducers from unstimulated sediments, and analyzed microbial community composition via 16S ribosomal RNA amplicon sequencing.
Our results demonstrated the in situ removal of V(V) (72-98%) from sediments with an organic carbon amendment, suggesting microbially mediated removal of V(V). In addition, MPNs showed up to a 50-fold increase in cell numbers (1,100,000 versus 23,000 cells/g of sediment) of organisms able to reduce V(V) in columns after three weeks of acetate addition. From unamended sediment we isolated a strain of Simplicispira (str. BDI) that grows well at high vanadium concentrations. Str. BDI was able to grow on nitrate and reduces vanadium. Ongoing genome sequencing and physiological experiments will target this organism’s physiology, and identify potential vanadium-reducing enzymes for which there is no information at present. Community analysis of the stimulated sediments indicates the presence of strain BDI. 16S rRNA sequencing shows that vanadium- and acetate-stimulated sediments exhibit lower richness and evenness than background sediments. Strain BDI was found in vanadium-addition sediments at a higher abundance than in acetate addition or background sediments (3% versus < 0.1%). These results suggest that vanadium reducers thrive in contaminated sediments, can be selected for by increasing vanadium concentrations and show that in situ microbial communities can be used to remove vanadium from groundwater for an extended period of time.

499A  Isolation of bacteria capable of degrading pharmaceuticals and personal care products to low ng/L concentrations
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Pharmaceutical and personal care products (PPCP) discharged with wastewater treatment plants (WWTP) effluents is an emerging surface water quality concern. Biological transformation has been identified as an important removal mechanism during wastewater treatment; however, bacteria capable of removing PPCP to ng/L and low µg/L concentrations observed in WWTP effluents have not yet been identified. Our research has focused on developing bioaugmentation technologies for improved removal of PPCP. For bioaugmentation, targeted bacteria must degrade PPCP in the presence of other carbon sources, grow on other carbon sources, maintain the ability to degrade PPCP when grown without the PPCP, and degrade PPCP to ng/L concentrations. As a first step, we isolated bacteria that meet these characteristics.

Isolation was achieved through a series of selective enrichment steps. Mineral media containing 1 mg/L PPCP as sole organic carbon source was inoculated with municipal activated sludge (1% or 10% by volume). After degradation of the PPCP was observed, the enrichment culture was transferred to fresh mineral media containing 1 mg/L PPCP (10% transfer by volume). Transfers continued until the activated sludge was diluted to approximately 10^-8 of the initial concentration. The enrichment was then plated on solid R2A media augmented with PPCP. Colonies with different morphologies were transferred into 1X R2A broth containing 1 mg/L PPCP to confirm degradation ability. 16S rRNA gene sequencing analysis was conducted to identify the isolated bacteria. PPCP concentrations were monitored using HPLC-UV (mg/L to µg/L concentration ranges) or LC-MS/MS (µg/L concentration range). PPCP concentrations in the ng/L concentration range were determined by solid phase extraction followed by LC-MS/MS. Bacterial growth was monitored by optical density.

Bacteria have been isolated that can degrade triclosan, bisphenol-A (BPA) and ibuprofen below 10 ng/L and naproxen, 17β-estradiol and gemfibrozil below 10 µg/L (testing is on-going to assess lower concentrations levels). Generally only one compound was degraded by each bacteria isolated. Seven of the eleven bacteria isolated were sphingomonads. Substrates high in proteins were able to be used by all bacteria and sugars were rarely used a growth substrate. Degradation of the contaminants generally occurred before the growth of the bacteria on other carbon sources. The effects of initial PPCP concentration and initial bacterial biomass on contaminant degradation kinetics have been studied for a BPA degrader, designated BD32. An initial BPA concentration in the low mg/L range resulted in a degradation rate between 0.15-0.25 L/mg-hr, whereas initial BPA concentrations in the µg/L range had degradation rates between 1.5-2.5 L/mg-hr for BD32. The BPA degradation rate also increased with increased BD32 biomass. When augmented to activated sludge, BD32 had a BPA degradation rate between 0.8-1.2 L/mg-hr with a µg/L initial BPA concentration.

These bacteria isolated meet the above criteria for bioaugmentation. This study has provided valuable information for future bioaugmentation experiments and advanced our knowledge of these bacteria capable of degrading PPCP to such low levels.
The elemental stoichiometry of marine plankton communities plays a key role in global biogeochemical cycles. However, studies on the stoichiometry of marine plankton have largely focused on autotrophic lineages, leaving the range and controls on the stoichiometry of heterotrophic bacteria poorly understood. Stoichiometry is controlled by a combination of taxonomic life history constraints and the physiological response to environmental conditions. Our objective was to estimate the range of carbon (C), nitrogen (N), and phosphorus (P) ratios and possible phylogenetic constraints on the elemental composition of ecologically relevant heterotrophic marine bacteria. To address this objective, we grew a diverse panel of heterotrophic marine bacteria in a “common garden” design to minimize environmental influence and isolate effects of phylogeny on cellular stoichiometry. Growth conditions were standardized using a light/dark cycling incubator and filtered seawater amended with carbon and nutrients in the Redfield ratio (C:N:P = 106:16:1). Cells were collected at late exponential to early stationary phase and analyzed for C, N, and P content. Elemental composition was measured for representatives from the Orders Alteromonadales, Bacillales, Oceanospirillales, Pseudomonadales, and Vibrionales. C:N content varied little across (mean C:N ranged from 4.1 to 5.1) or within taxonomic groups, except for in the Bacillales, where two representatives differed in their C:N ratio by 1.7. Mean N:P and C:P ratios were more variable across taxa, ranging from 9.8-20.2 and 44.2-93.5, respectively. C:P ratio was most variable, particularly among representatives of Alteromonadales (39.6-48.8), Vibrionales (69.6-84.2), and Bacillales (31.6-71.7). The variation in elemental ratios detected within taxonomic groups of heterotrophic marine bacteria suggests that phylogenetic constraints on cellular stoichiometry can be quite flexible. Furthermore, our results suggest that the C:N and C:P ratios of heterotrophic marine bacteria are lower than the Redfield ratio. The discrepancy between the observed stoichiometry of heterotrophic marine bacteria and the Redfield ratio carries implications for predicting interactions among major biogeochemical cycles and suggests that the ratio of heterotrophic to autotrophic plankton biomass is important for the overall stoichiometry of surface communities.