



## Challenges encountered calibrating N<sub>2</sub>O dynamics from mixed cultures

**Domingo Felez, Carlos; Pellicer i Nàcher, Carles; Petersen, Morten S.; González-Combarros, Rosana; Jensen, Marlene Mark; Sin, Gürkan; Smets, Barth F.**

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# Poster Abstracts

## TOPIC 1: NITROGEN CYCLE ECOGENOMICS

### POSTERS:

[EG-1] *also a workshop presentation*

### **Phylogeny, diversity and evolution of the archaeal ammonia monooxygenase subunit A – a framework for classification and ecological analysis of ammonia-oxidising archaea**

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Ammonia-oxidising archaea (AOA) are ubiquitous in most environments and known to be major players in the global nitrogen cycle. Over the past decade, environmental studies of ammonia oxidisers have greatly relied on amoA genes, encoding a subunit of the ammonia monooxygenase in bacteria and archaea, making it the second most sequenced marker gene after the 16S rRNA gene. The archaeal variant alone comprises ~55% of all amoA gene sequences deposited in public databases. However, phylogenetic analysis and classification of amoA genes, namely of AOA, is notoriously difficult, as shown by the general inconsistency and low statistical support among most published phylogenies. In fact, current AOA amoA gene phylogenies only reliably resolve broad order-level lineages, consistent with those previously known based on 16S rRNA genes. We inferred a robust phylogeny based on thorough analyses of ~45.000 available archaeal amoA gene sequences, and show that the global gene diversity is represented by several distinct lineages spanning different phylogenetic levels. We discuss major bottlenecks that likely precluded more consistent and resolved phylogenies in previous studies. The phylogeny reflects broad environmental distribution patterns, although the environmental diversity is unevenly distributed, with both broad and narrow clades being either cosmopolitan or ecosystem-specific. Moreover, some clades harbour molecular signatures, such as distinct GC contents, which, to some extent, are also associated with specific environmental niches. Our results synthesize the archaeal amoA gene information obtained over 10 years and will serve as a phylogenetically-informed framework to classify and characterise the ecology of AOA in a reproducible way.

[EG-2]

## Characterization of *Nitrosomonas eutropha* D23 as a human skin probiotic

**Neeraja Vajrala**<sup>1</sup>, Ioannis Gryllos<sup>2</sup>, Colleen Cutcliffe<sup>3</sup>, James Bullard<sup>3</sup>, John Eid<sup>3</sup>, Larry Weiss<sup>2</sup>, David Whitlock<sup>2</sup>, Spiros Jamas<sup>2</sup>, Luis Sayavedra-Soto<sup>1</sup>  
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The development of skin probiotics based on ammonia-oxidizing bacteria (AOB) is currently an emerging area of research. It has been proposed that these bacteria when applied topically have the potential to improve skin condition, as ammonia oxidation products, nitrite and nitric oxide, may reduce skin inflammation, promote blood vessel relaxation and wound healing. Importantly, these bacteria generate energy exclusively from ammonia, but not from organic sources, and have never been linked to animal or human infection. Here, we describe the characterization of *Nitrosomonas eutropha* D23, a strain currently under evaluation as a natural biological delivery system of nitrite and nitric oxide to the skin.

*N. eutropha* D23, a novel previously uncharacterized strain, was initially enriched in batch culture from organic farm soil, then self-applied onto healthy human skin for up to several months. Microbial samples collected by swabbing the treated skin were then cultured in media containing ammonium, carbonate, and mineral salts. Axenic *N. eutropha* D23 cultures were subsequently obtained from isolated colonies grown on nylon disk membranes placed onto basal medium-soft-agarose plates for three weeks. To achieve reproducible yields at high cell densities, growth of *N. eutropha* D23 was optimized in steady-state chemostat culture (OD ~0.5 at 600nm). Harvested cells from this system remained viable upon storage at 4 °C for up to 12 months, generating consistent levels of nitrite upon subculture in batch conditions.

The *N. eutropha* D23 genome encompasses a single 2.54 Mb chromosome and is the smallest amongst all published genomes of AOB to date, including those of *N. eutropha* C91 and *N. europaea* ATCC 19718. The draft genome contains 2724 candidate protein-coding sequences, in addition to 41 tRNA genes and a single 16S-23S-5S rRNA operon. Initial analyses revealed over 99% identity of the *N. eutropha* D23 rRNA gene sequences with those from *N. eutropha* C91, and also identified approximately 160 unique genes in the D23 chromosome. Further *in silico* whole genome screening confirmed the absence of undesirable toxin and virulence factor genes. Additional testing *in vitro* indicated susceptibility of *N. eutropha* D23 to macrolides (erythromycin) and aminoglycosides (gentamicin) and no expression of beta-lactamases, at least under the conditions tested. Further studies are underway to explore the potential of AOB as skin probiotics, as well as their therapeutic applications in the treatment of skin inflammatory conditions.

[EG-3]

## ***Nitrosomonas europaea* responses at suboptimum carbonate levels in continuous culture**

**Brett L. Mellbye**,<sup>1</sup>, Peter J. Bottomley,<sup>2</sup> and Luis A. Sayavedra-Soto,<sup>1</sup>.

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*Nitrosomonas europaea* is a chemolithoautotrophic bacterium that uses NH<sub>3</sub> and CO<sub>2</sub> for growth. We interrogated the responses of *N. europaea* in 60 mM NH<sub>4</sub><sup>+</sup> to replete (~30 mM) and suboptimum levels (1.0 mM and 0.2 mM) of carbonate in continuous steady-state culture. In replete carbonate medium the cells consumed 60 mM NH<sub>4</sub><sup>+</sup> and reached an OD<sub>600</sub> of 0.2. In 1.0 and 0.2 mM carbonate medium, the cells consumed 25 mM NH<sub>4</sub><sup>+</sup> and both treatments grew to an OD<sub>600</sub> of approximately 0.07. This result indicates the air supply to the chemostat supplemented CO<sub>2</sub> to the 0.2 mM carbonate culture, but was insufficient to significantly increase the C supply and cell density of the 1.0 mM carbonate culture. Transcriptomic analysis showed differential expression ( $p \leq 0.05$ ) of 435 genes between replete and 1 mM carbonate treatments, and of 127 genes between 1 mM and 0.2 mM carbonate treatments. Higher expression of genes encoding RuBisCo, cbb pathway enzymes, carbonic anhydrase (NE1926), ammonium transporter (Rh50), and nitrite (nirK) and nitric oxide (norQ) reductases were observed in 1.0 mM carbonate, but expression of these genes decreased in 0.2 mM compared to 1.0 mM carbonate. Chaperonin and cold-shock protein mRNAs were also more abundant in 1 mM carbonate while those for electron transport and several multicopper oxidases decreased in abundance. The changes in expression in 0.2 mM compared to 1.0 mM carbonate suggest adaptations to low carbonate in the medium, and perhaps reflect a greater reliance on atmospheric CO<sub>2</sub> to meet the C requirements of *N. europaea*.

[EG-4]

## **Comparative genomic analysis of phylogenetically distant two *Nitrospira* strains isolated from a wastewater treatment plant**

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Nitrite oxidation is a key step of biological nitrogen removal in wastewater treatment plants (WWTPs). In most WWTPs the genus *Nitrospira* represents the dominant nitrite-oxidizing bacteria (NOB) and is affiliated with lineage I or II. Recently, phylogenetic analysis based on 16S rRNA gene or *nxB* gene, and ecophysiological approaches such as MAR-FISH have revealed high diversity of *Nitrospira* belonging to two lineages in WWTPs, and distinct ecological differentiation including affinity and sensitivity for nitrite, utilization of organic compounds such as formate, and interaction with ammonia oxidizers and heterotrophs. Here, we report comparative genomic analysis of two *Nitrospira* pure strains (ND1 and NJ1 belonging to lineages I and II, respectively), which were previously isolated from a WWTP by our research group. The draft genomes of both strains contain a locus with genes coding urease subunits, and ureolytic activity was confirmed by physiological experiments. Additionally, the strain NJ1 possesses 4 copies of ammonium permease (transporters), and is likely to utilize ammonium for nitrogen assimilation, considering that 1 or 2 copies were found on genomes of NOB available in database. Unexpectedly, although most nitrifying bacteria possess ability of motility such as flagella and chemotaxis, draft genome of the strain NJ1 lacks most genes involved motility. Therefore, a genome comparison of *Nitrospira* strains provides a genetic basis to understand physiological difference, which is consistent with functional and ecological diversity of *Nitrospira* in WWTPs.

[EG-5]

## **Enrichment, isolation, genome-sequencing and growth physiological characterization of D1FHST, the recovered type strain of *Nitrosococcus nitrosus*, the valid type species of the genus *Nitrosococcus***

**Lin Wang**<sup>1</sup>, Chee Kent Lim<sup>1</sup>, Hongyue Dang<sup>2</sup>, and Martin G. Klotz<sup>1, 2, 3</sup>

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Jiaozhou Bay is a hypereutrophic semi-closed water body hosting the metro area of Qingdao, China and one of the largest petroleum shipping hubs in Asia. Due to an increasing input of crude oil, industrial pollution, agricultural fertilized water and human wastewater, the environmental quality of Jiaozhou Bay (JB) has dramatically deteriorated in recent decades with one of the consequences being the high concentration of fixed nitrogen in the Bay. This unfolding environmental tragedy has motivated many biogeochemical and ecological research projects, one of which is the spatial and temporal study of the sediment bacterial community including ammonia-oxidizing microbes (AOM) such as aerobic chemolithotrophic bacteria and Thaumarchaeota and anammox bacteria. Attempts to enrich AOM from JB sediments sampled during a mini cruise in September 2008 succeeded in the isolation into pure culture of a strain, D1FHS, that was phylogenetically most closely related to *Nitrosococcus halophilus* Nc4. A high-quality genome sequence was created using PacBio-based high-throughput sequencing that included relevant genetic markers of other *Nitrosococcus* genomes. The average nucleotide identity (ANI) calculated for the genome of D1FHS and *N. halophilus* Nc4 is 89.30%, which is significantly below the cut-off for the delineation of species. Given existing historic descriptions and that fact that the no strain of the validly described type species of the genus *Nitrosococcus*, *Nitrosococcus nitrosus*, remained in culture since 1912, we decided to designate D1FHS as the type strain of *Nitrosococcus nitrosus*. The optimum culture condition for *N. nitrosus* D1FHST is pH 7.0~7.5 with a salt concentration of 700 mM at 28°C in dark. Genome analysis and physiological studies revealed that *N. nitrosus* D1FHST is more closely related to *Nitrosococcus halophilus* Nc4 than to characterized *Nitrosococcus oceanus* and *N. watsonii* strains.

**[EG-6] also a session presentation**

## **Proteo-Genomics of *Nitrososphaera viennensis***

**Christa Schleper**

*Department of Ecogenomics & Systems Biology, University of Vienna, Austria*

The soil thaumarchaeote *Nitrososphaera viennensis* has become a well studied model organism for ammonia oxidizing archaea. With 2.5 Mb and 3123 predicted genes, the *N. viennensis* genome is similar in size to the other group 1.1b genomes (mostly soil organisms) and considerably larger than most genomes of group 1.1a (mostly marine strains). I will present the analysis of its genome and proteome with respect to energy metabolism and ammonia assimilation and their regulation and will discuss (eco-)physiological insights in comparison to its closest relatives, and in contrast to the more streamlined genomes of the marine organisms and the proteome of *Ca. Nitrosopelagicus brevis*.

[EG-7]

## **Distributed metabolism in suspended and attached growth anammox bioreactors revealed through metagenomic sequencing**

**Christopher E. Lawson**<sup>1</sup>, Sha Wu<sup>2</sup>, Ananda S. Bhattacharjee<sup>2</sup>, Katherine D. McMahon<sup>1,3</sup>, Ramesh Goel<sup>2</sup>, Daniel R. Noguera<sup>1,4</sup>

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Anaerobic ammonium oxidation (anammox) represents one of the most innovative biotechnologies for energy-efficient nitrogen removal from wastewater. Despite its ubiquity and industrial importance, microbial interactions driving cooperation and competition between anammox bacteria and neighboring microorganisms in engineered ecosystems remain poorly understood. Elucidating these and other ill-defined reactions is essential for ecosystem modeling of anammox processes and for identifying metabolic constraints that limit process performance.

Here, we use metagenomic sequencing and differential coverage binning to recover population genomes from two lab-scale anammox bioreactors employing suspended and attached growth modes. We reveal that anammox populations recovered from the bioreactors were metabolically versatile and exhibit novel opportunities for metabolic cooperation with neighboring microorganisms through aromatic compound degradation, single-carbon compound metabolism, and inorganic nutrient cycling.

## **TOPIC 2: NITROGEN CYCLE ECOLOGY & NICHE DIFFERENTIATION**

**POSTER & TALK: Monday Session, 5-6PM CCIS L1-140**

[EN-1]

### **Response of soil nitrification to temperature gradient shifts**

**Anne Taylor**<sup>1</sup>, Andrew Giguere<sup>1</sup>, David Myrold<sup>1</sup>, and Peter Bottomley<sup>1,2</sup>

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Nitrification potential (NP) activities were measured in pairs of cropped and noncropped soils from four sites in Oregon using octyne to discriminate between the contributions of AOA and AOB to nitrification over a range of temperatures (4 – 42°C). Several general trends were observed. Regardless of soil type, AOA consistently expressed their maximum NP rate between 30 and 37°C, contributing between 63 and 100% of the total NP. In contrast, AOB achieved their maximum NP rate at 16 or 23°C where they contributed 66 to 94% of the total NP. At even lower temperatures (4 - 10 °C) AOB dominated nitrification and contributed an average of 75% of the total NP. Despite the similar temperature optima, the profiles of activity by AOA or AOB across the temperature range differed among the soils, and the intervals where NP by either AOA or AOB first responded significantly to temperature increases were different. The temperature coefficient  $Q_{10}$  calculated over these intervals were often larger than might be expected if the upturn in NP was due only to increases in enzyme activity, and led us to hypothesize that subgroups of  $\text{NH}_3$  oxidizers were contributing to the NP over different and overlapping temperature ranges. Further experiments are being carried out to refine the temperature profiles of AOA and AOB  $\text{NH}_3$ -oxidizing activities compared with their biosynthetic potential to recover and/or sustain  $\text{NH}_3$ -oxidizing activity, and to determine if subgroups of ammonia oxidizers are active over different temperature ranges.

[EN-2]

## **Surface Soil as an Extreme Environment: Diurnal Temperature Swings Impact Dynamics of N-fertilizer Amendments to Soil**

**R.A. Sanford<sup>1</sup>**, J.C. Chee-Sanford<sup>2</sup>, L. Orellana<sup>3</sup>, S.A. Napieralski<sup>1</sup>, S. G. Chee<sup>4</sup>, and F. Löffler<sup>5</sup>

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Seasonal differences in soil temperature have always been recognized, yet diurnal soil temperature change and maximums are not commonly implicated as significant drivers of microbial communities and activities. We measured temperatures exceeding 50°C with diurnal delta-T exceeding 20°C in the top 5 cm of agricultural soil April through June of each year. We hypothesized that daily surface temperature variation acts as a fundamental driver for microbial activity and niche differentiation of the microbial community composition. To evaluate our hypothesis, triplicate aerobic microcosms amended with urea (5 mM)+NH<sub>4</sub>NO<sub>3</sub> (5 mM) or no N were constructed using saturated surface soil from two Illinois sites: Agricultural (Ag) and prairie (Pr). Closed microcosms were incubated at 25°C, 35°C, and diurnal cycle 20°-35°C. Diurnal-incubated Ag-soil showed the most rapid NH<sub>4</sub><sup>+</sup> depletion and N<sub>2</sub>O generation, which occurred after a lag of 7 days. In contrast, Pr-soil exhibited a large initial N<sub>2</sub>O flux in the first 24h when diurnally incubated, with 3X the mass release of the Ag-soil: 15.4 μmoles vs. 4.2 μmoles, respectively. Nitrite accumulated to 1 mM in the diurnally incubated Pr-soil at 24h. Ag-soil did not show any initial nitrite accumulation. Microbial community analyses using 16S rRNA- and functional genes showed differences dependent on incubation temperatures, however the greatest deviation in alpha- and beta-diversity was observed at 35°C. Results confirm diurnal temperature conditions yield significantly different activity measurements than obtained with static temperature conditions, suggesting daily temperature cycles should be considered for assessment of soil microbial communities and function.

[EN-3]

## **The predominant role of ammonia-oxidizing archaea in acid soils and its responses to environmental perturbation and climate change**

**Hang-Wei Hu**, Ji-Zheng He

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Acid soils are globally distributed and occupied approximately 30% of the world's total ice-free lands, however, the functionally dominant nitrifiers and underlying mechanisms for acid soil nitrification are a long-standing mystery. Previous studies suggest that ammonia-oxidizing archaea (AOA) would be more adapted to ammonia-limited oligotrophic conditions, which seems to be favored by protonation of ammonia, turning into ammonium in low-pH environments. In this study, we investigated the autotrophic nitrification activity of AOA and ammonia-oxidizing bacteria (AOB) in five acidic soils (pH<4.50) during microcosm incubation for 30 days. <sup>13</sup>C<sub>CO2</sub>-DNA-stable isotope probing results showed significant assimilation of <sup>13</sup>C-labeled carbon source into the amoA gene of AOA, but not of AOB. Addition of the nitrification inhibitor dicyandiamide (DCD) completely inhibited the nitrification activity and CO<sub>2</sub> fixation by AOA, accompanied by decreasing AOA amoA gene abundance. AOB amoA gene abundance decreased in all microcosms irrespective of DCD addition, and mostly showed no correlation with nitrate concentrations. Phylogenetic analysis revealed active <sup>13</sup>C<sub>CO2</sub>-labeled AOA belonged to groups 1.1a-associated and 1.1b. Taken together, these results provided strong evidence that AOA have a more important role than AOB in autotrophic ammonia oxidation in acidic soils. These findings are further supported by the increasing metabolic activity of AOA in acid soils under environmental perturbations like irrigation and fertilization. Moreover, AOA were found to respond more strongly to climate warming than to elevated CO<sub>2</sub>, and AOA tended to dominate the nitrification in acid soils under future climate warming.

[EN-4] *also a workshop presentation*

## **Uncoupling of ammonia oxidation from nitrite oxidation, and its impact upon nitrous oxide production in a grassland soil**

**Andrew Giguere**<sup>a</sup>, Anne Taylor<sup>a</sup>, David Myrold<sup>a</sup>, Peter Bottomley<sup>a,b</sup>

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Ammonia oxidation is typically thought of as the rate limiting step in nitrification. However, in NH<sub>4</sub><sup>+</sup> supplemented aerobic soil slurry incubations of native grassland soils from semiarid eastern Oregon an uncoupling of NH<sub>4</sub><sup>+</sup> oxidation from NO<sub>2</sub><sup>-</sup> oxidation was observed. The accumulation of NO<sub>2</sub><sup>-</sup> was transient, and NO<sub>2</sub><sup>-</sup> concentrations increased from 0-12 h, and persisted for 48 h. From 12-48 h, NO<sub>2</sub><sup>-</sup> levels declined, while the NH<sub>4</sub><sup>+</sup> oxidation rates remained linear over 48 h. The increase in NO<sub>2</sub><sup>-</sup> oxidizing potential was prevented by bacterial protein synthesis inhibitors, demonstrating that while NH<sub>4</sub><sup>+</sup> oxidizers are able to oxidize NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> immediately upon addition of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> oxidizers require de novo protein synthesis to achieve their maximum NO<sub>2</sub><sup>-</sup> oxidation potential. N<sub>2</sub>O production was also measured, and was positively and linearly correlated with the NO<sub>2</sub><sup>-</sup> concentration that accumulated over 24 h. Furthermore, our data suggests that N<sub>2</sub>O production is directly dependent on nitrification activity. N<sub>2</sub>O production was completely inhibited by low acetylene concentrations (0.02%), and was not stimulated by high acetylene concentrations (10%). Even during the period after maximum NO<sub>2</sub><sup>-</sup> accumulation, acetylene prevented N<sub>2</sub>O production, further suggesting that NH<sub>4</sub><sup>+</sup> oxidation accounted for N<sub>2</sub>O production. Our data demonstrate that NH<sub>4</sub><sup>+</sup> dependent N<sub>2</sub>O production from nitrification can be brought about by the temporary uncoupling of NH<sub>4</sub><sup>+</sup> oxidation from NO<sub>2</sub><sup>-</sup> oxidation. Further studies are underway to identify what factors affect the magnitude of the uncoupling of NH<sub>4</sub><sup>+</sup> oxidation from NO<sub>2</sub><sup>-</sup> oxidation.

**[EN-5] also a workshop presentation**

## **Nitrification in the eastern tropical North and South Pacific OMZs**

**Xuefeng Peng**(1), Clara Fuchsman(2), Amal Jayakumar(1), Allan Devol(2), and Bess Ward(1)

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Nitrification, the oxidation of ammonium to nitrite and nitrate, produces the substrates for denitrification, thus fueling the anaerobic nitrogen loss processes in oxygen minimum zones (OMZs). Incubations with  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_2^-$  were performed to measure ammonium and nitrite oxidation on cruises in the eastern tropical North (March/April 2012) and South (July 2013) Pacific (ETNP and ETSP, respectively) OMZs. We investigated the depth distribution of both processes, as well as their sensitivities to substrate concentration and light.  $^{15}\text{NO}_2^-$  and  $^{15}\text{NO}_3^-$  production were determined using isotopic ratio mass spectrometry, with the azide and the denitrifier methods, respectively. Both archaeal and betaproteobacterial *amoA* genes were quantified using qPCR. Subsurface maxima of both rates and *amoA* gene abundances were consistently found in the oxycline above the anoxic layer in both OMZs. The observed tight correlation between ammonium oxidation and nitrous oxide concentration in the oxycline suggests that ammonium oxidation was an important source of nitrous oxide. At anoxic depths, substantial number of *amoA* genes were detected, but ammonium oxidation were undetectable or negligible, whereas nitrite oxidation rates were sometimes high. At an offshore station in the ETSP, ammonium oxidation displayed an extremely high affinity for ammonium, with a half-saturation concentration of 27 nM. At 10% surface irradiance, ammonium oxidation rates were detectable but lower than in the dark. No significant effect of light on nitrite oxidation was found. These results indicate a highly dynamic internal nitrogen cycling in OMZs, while the high rates of nitrite oxidation at anoxic depths remain a conundrum.

[EN-6] *also a workshop presentation*

## **Differences in nitrous oxide yield from bacterial- and archaeal-driven soil nitrification**

**Linda Hink** (a), Graeme W. Nicol (a, b) and James I. Prosser (a)

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Ammonia oxidisers are the major contributors to nitrous oxide (N<sub>2</sub>O) emissions from fertilised oxic soils, where classical denitrifiers play a minor role. Predictions of ammonia oxidiser-driven emissions solely refer to bacterial ammonia oxidisers (AOB), but it was recently discovered that archaea (AOA) that contribute significantly to ammonia oxidation in soil produce N<sub>2</sub>O in laboratory cultures. Nothing is known of relative contributions of AOA and AOB to soil N<sub>2</sub>O emissions. In this study, we tested the hypothesis that AOB dominate ammonia oxidation in ammonium-fertilised soils, and thereby dominate N<sub>2</sub>O emissions, but that AOA are significant N<sub>2</sub>O emitters in non-fertilised soils. The effects of ammonium amendment on nitrification, N<sub>2</sub>O production, growth and transcriptional activity by AOA and AOB were investigated in oxic soil microcosms, using 1-octyne, a proposed specific inhibitor of AOB, to discriminate AOA and AOB activity. N<sub>2</sub>O production was directly related to ammonia oxidation, rather than denitrification, was stimulated by ammonium amendment and was reduced by 1-octyne addition. 1-octyne specifically inhibited AOB growth, activity and N<sub>2</sub>O production. AOA dominated oxidation of ammonia derived from mineralisation as indicated by similar results in control and 1-octyne-treated soils. Greater stimulation of AOA following ammonium amendment with 1-octyne suggested that AOA compete with AOB for ammonia. Restriction of N<sub>2</sub>O production to the chemical reaction of nitrification intermediates in AOA was consistent with a N<sub>2</sub>O yield half that of AOB possessing additional biological mechanisms for N<sub>2</sub>O production. The results support our hypothesis and suggest potential mitigation strategies for N<sub>2</sub>O emissions by fertilisation methods favouring AOA.

[EN-7]

## Dominance of AOA than AOB in Acidic Forest Soils in Subtropical China

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Recent discovery on ammonia-oxidizing archaea (AOA) expands ammonia oxidation in addition of ammonia-oxidizing bacteria (AOB), but little is known about AOA community structure and abundance in subtropical forest soils. In this study, both AOA and AOB (collectively ammonia-oxidizing microorganisms, AOM) were investigated in eight forests at different soil depths in Nanling National Nature Reserve in subtropical China, including 1 mountainous dwarf forest, 2 bamboo forests, 2 evergreen broadleaf forests, and 3 coniferous and broadleaf mixing forests. Nutrients (organic carbon, NH<sub>4</sub> and total phosphorus) and pH of the soil samples were analyzed to study their relationship with AOM. Our results showed that the forest soils (pH 4.24-5.10) harbored a wide range of AOA phylotypes, including genera *Nitrosotalea*, *Nitrososphaera*, and additional 6 clusters, one of which was the first time reported. For AOB, only members of *Nitrosospira* were retrieved. Moreover, AOA dominated over AOB in terms of *amoA* gene abundance in most soils (13/16). Principal coordinates analysis showed that soil depth was an important factor shaping the community structure of AOM. AOA diversity and abundance in low layers were higher than surface layers while AOB had an opposite trend. The diversity of AOA was positively correlated with pH, and the abundance of AOA was positively correlated with available phosphorus. However, the diversity and abundance of AOB were not significantly correlated with any soil parameters. The results suggest that AOA probably played a more important role than AOB in acidic forest soils in subtropical China.

[EN-8]

## **Development of a synthetic microbial community towards understanding nitrification and interactions among bacteria, fungi and plants interaction in soil**

**Xue Jiang**

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To understand the potential interactions between plant-associated fungi and plants with soil microbial nitrification community, there have been attempts of monitoring entire soil microbial nitrification community with or without plants, or plant mutualistic fungi. These studies are challenging, due to huge complexity of soil composition and microbiota, which make it difficult to reach a consensus on whether any group of organisms (plant, fungi or bacteria) actively manipulates the soil microbial communities and in particular the nitrifying microbes.

Here, we aim to overcome this difficulty by constructing a model system that would be tractable to analyse plant-fungi-microbe interactions. To this end, we are developing a synthetic bacteria-fungi-plant system to understand the dynamics of the nitrification community. By choosing certain species to represent key characters, and culturing them together in a defined medium, we could simulate the soil nitrification. This model system would allow development and testing of hypotheses of cross-kingdom interactions among soil organisms. For example, by including a plant mutualist fungus *P.indica* into the model system, we could observe how nitrification process (i.e. microbial component) would be affected by its presence. Further more, adding a plant into this system could show us what kind of influence plant could bring to the system.

We have established media and growth conditions for co-culture nitrosomonas and nitrobacter, and interfacing this co-culture with *P.indica*. In parallel to ongoing experimental work, we have developed a mathematic model of the nitrosomonas-nitrobacter co-culture to study the potential effects of thermodynamic inhibition on the dynamics of this system.

[EN-9]

## **Molecular and isotopic analyses reveal high nitrification activity performed by ammonia-oxidizing bacteria in a deep oligotrophic mountain lake**

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*CREAF & CEAB-CSIC*

Nitrification links the two main forms of reactive nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) that impact remote ecosystems through N increase in atmospheric deposition. In the Pyrenees, the balanced proportion of ammonium and nitrate (1:1) in the deposition is not found in stream and lake waters. Nitrate is usually much higher than ammonium (~10:1) suggesting an important role of nitrification. We sampled monthly during an annual cycle a deep and oligotrophic mountain lake (Lake Redon) at 5 depths (2, 10, 20, 35 and 60 m) and complemented the ordinary chemical analysis with isotopic ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ ;  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$ ) and molecular (bacterial and archaeal amoA genes copies determined by quantitative real-time PCR amplification) analyses. Concentrations changes coupled with the enrichment of ammonium  $\delta^{15}\text{N}$  and the depletion of nitrate  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  indicates high nitrification activity. Nitrification mostly occurred in the low-oxygen deep waters during the ice-covered period. The oxidation of ammonium to nitrite (the rate limiting step of nitrification) was performed mainly by Bacteria and the bacterial amoA gene reached more than  $10^5$  copies·ml<sup>-1</sup>.

[EN-10]

## **Nitrogen transformations in lake sediments receiving nitrate-rich waste water input**

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European commission has obliged Finland and other Baltic states to reduce nitrate load, which requires high investments on nitrate removal processes and may increase emissions of greenhouse gases, e.g. N<sub>2</sub>O, in waste water treatment plants (WWTPs). We are currently testing whether spatial optimization of the waste water discharge offers a novel economically feasible method for nitrogen (N) removal, increasing both the area and time that nitrified waste water will be in contact with the reducing microbes of the sediment in the recipient lake, thus enhancing microbial-driven N transformation rates. We are utilizing stable isotope labelling methods to follow seasonal changes in denitrification, nitrous oxide formation and/or dissimilatory nitrate reduction to ammonium (DNRA) in the lake sediments receiving nitrate-rich waste water input. We have also investigated the connections between observed process rates and microbial community composition by using next generation sequencing and ddPCR of nirS, nirK, nosZ, and nrfA genes. Before spatial optimization, we found highest N process rates near the WWTP outlet pipe, where high nitrate concentrations promoted denitrification, but unfortunately also N retention through DNRA. However, we observed strong seasonal patterns in the process rates, reflecting the mixing patterns of the waste water with the lake water.

[EN-11]

## **Characterization of nitrogen cycle involved in nitrous oxide emissions in High Arctic polar desert**

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Nitrous oxide (N<sub>2</sub>O) is a greenhouse gas with strong global warming potential and is the most serious ozone-depleting substance in this century. With these properties, N<sub>2</sub>O emission from Arctic soil is a great concern about maintenance of Arctic ecosystem specialized for the cold temperature and other unique characteristics of the environment. In High Arctic, nitrification was shown to be the dominant microbial process of N<sub>2</sub>O production (Siciliano et al., 2009). Although the major metabolic step for N<sub>2</sub>O production is widely known, how nitrifier interacts with critical edaphic and environmental factors to produce N<sub>2</sub>O in the region is not clearly characterized. We hypothesized that cryoturbation and soil properties including soil pH and soil nutrient contents, influence nitrifier to determine N<sub>2</sub>O emission level. Study site was located at Alexandra Fjord, Ellesmere Island, Nunavut Territory (78°53'N, 75°55'W). The site had two different types of desert with being different pH level from each other. Frost boils was a large component of the landscape, and some frost boils were affected by a type of cryoturbation, diapir formation in both deserts. Gross rates of nitrification and mineralization were measured using <sup>15</sup>N isotope-dilution techniques (Hart et al. 1994) and were compared between diapir frost boils and non-diapir frost boils across the two deserts. While both gross nitrogen transformation rates were significantly different between the two deserts, neither of them was between the frost boils with and without diapir formation. To assess if there is the relationship between nitrifier and the other factors, soil properties are being analyzed.

[EN-12]

## **Ammonia manipulates the ammonia oxidizing archaea and bacteria in the coastal sediment-water microcosms**

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Background: Ecological studies of ammonia oxidizing microorganisms and physiological study of the first isolated ammonia oxidizing archaea (AOA) strain have proposed an acceptable hypothesis that AOA may contribute more to nitrification in environments with low ammonia concentration but ammonia oxidizing bacteria (AOB) may be more favorable in environments with high ammonia concentration. Evidence for the above hypothesis have been provided by lab simulating microcosm studies for soil environment. Unfortunately in aquatic environments, the hypothesis has not been confirmed yet in laboratory cultivation system. Methods: A series of coastal sediment-water microcosms with different ammonia concentrations were set up and cultivated for 56 days, in which DNA-SIP was applied in two microcosm groups with 0.1 and 20 mg NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup>. Quantitative PCR (qPCR), quantitative reverse transcription PCR (RT-qPCR), and 454 pyrosequencing were applied to track precisely the archaeal and bacterial amoA genes. Results: The ratio of transcribed AOB amoA gene/AOA amoA gene increased from 0.1 to 43 as NH<sub>4</sub><sup>+</sup>-N increased from less than 0.1 mg L<sup>-1</sup> to 12 mg L<sup>-1</sup>, and AOA amoA transcription was undetected under 20 mg NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup>. The incubation of SIP microcosms revealed a faster <sup>13</sup>C-NaHCO<sub>3</sub> incorporation rate of AOA amoA gene under 0.1 mg NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup>, and a sole <sup>13</sup>C-NaHCO<sub>3</sub> utilization of the AOB amoA gene under 20 mg NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup>. Conclusions: Our results provided solid evidence on that AOA prefers to live and perform higher amoA transcription activity than AOB in ammonia-limited water environments; and AOB tends to take the first contributor place in ammonia-rich ones.

[EN-13]

## **PCR-based community analysis of methane-producing and metabolizing archaea and bacteria in the northern South China Sea and the coastal Mai Po Nature Reserve**

**Zhichao Zhou**, Ji-Dong Gu

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Community structures representing by *mcrA* gene based on 12 clone libraries from nSCS showed separate clusters indicating niche specificity, while, Methanomicrobiales, Methanosarcinales clade 1, 2 and Methanomassiliicoccus-like groups of methanogens were the most abundant groups in nSCS sediment. Novel clusters specific to the SCS were identified and the phylogeny of *mcrA* gene-harboring archaea was updated. Quantitative PCR was used to detect *mcrA* gene abundance in all samples: similar abundance of *mcrA* gene in the surface layers of mangrove ( $3.4\sim 3.9\times 10^6$  copies per gram dry weight) and of intertidal mudflat ( $5.5\sim 5.8\times 10^6$  copies per gram dry weight) was observed, but higher abundance ( $6.9\times 10^6$  to  $1.02\times 10^8$  copies per gram dry weight) was found in subsurface samples of both sediment types. MOB were more abundant in surface layers ( $6.7\sim 11.1\times 10^5$  copies per gram dry weight) than the subsurface layers ( $1.2\sim 5.9\times 10^5$  copies per gram dry weight) based on *pmoA* gene. Mangrove surface layers harbored more abundant *pmoA* genes than intertidal mudflat, but less *pmoA* genes in the subsurface layers. Meanwhile, in surface layers of all samples, more *pmoA* gene copies were detected than the subsurface layers. Reedbed rhizosphere exhibited the highest gene abundance of *mcrA* gene ( $8.51\times 10^8$  copies per gram dry weight) and *pmoA* gene ( $1.56\times 10^7$  copies per gram dry weight). This study investigated the prokaryotic communities responsible for methane cycling in both marine and coastal wetland ecosystems, showing the stratification of *mcrA* and *pmoA* gene diversity and abundance in the Mai Po Nature Reserve.

[EN-14]

## Microbial Characterization of Green Roof Soil

**Rebecca Ferrell**, Mary Culkin and Alec Curtis

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Green roofs are increasingly popular but relatively little research has been done into their microbial soil ecology. This study focuses on a green roof planted with *Sedum* spp. on the Student Success Building of the Auraria Campus in downtown Denver, Colorado. Four zones were designated: (A) shaded by walls; (B) additional reflected light; (C) direct sunlight and (C) no vegetation. Two ground-level control plots were also included. In this second year of the study we sought to: (1) quantify organic material in soils, (2) determine relative levels of soil Bacteria and Archaea, and (3) examine nitrification potentials. Organic matter (OM) content was evaluated by incineration, showing 5-8% OM in the 3 vegetative roof areas and 10-11% in ground level controls. The no-vegetation roof area had <1% OM (compost typically shows >20% OM). DNA was extracted using spin columns (PowerSoil, MoBio, Inc); soil DNA extractions conducted in parallel yielded 2-3 times more DNA per 0.25 g from ground-level plots compared with the vegetated roof zones. Quantitative PCR of DNA from vegetated roof plots using primers for small subunit ribosomal genes showed more than 2-fold higher Bacterial 16S rRNA sequences compared with 16S rRNA genes of Archaea; this trend was reversed in the non-vegetated region of the roof, where Archaeal signal prevailed. Nitrification potentials were observed by inoculating media containing ammonia (NH<sub>3</sub>) as the sole energy source with soil. Roof soils showed virtually no oxidation of ammonia over 2 weeks, while ground-level control soils showed evidence of nitrification with 48 hours.

[EN-15]

## **Nitrification Kinetics and Temperature Response of Ammonia-Oxidizing Bacteria and Archaea in an Agricultural Soil under Contrasting N Fertilization**

**Yang Ouyang<sup>1</sup>**, Jeanette M Norton<sup>1</sup>, Jennifer Reeve<sup>1</sup>, John Stark<sup>2</sup>, Mussie Habteselassie<sup>3</sup>

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The first step of nitrification in soils, ammonia oxidation to nitrite, is mediated by ammonia oxidizing bacteria and archaea (AOB and AOA). However, our understanding of the relative contribution of AOA and AOB to overall nitrification in the soil environment is limited. Our goal was to determine kinetics and temperature sensitivity for nitrate production in agricultural soils with different histories of nitrogen (N) fertilization. Soils were collected from corn field plots after four years of contrasting N treatment: control (no additional N), ammonium sulfate (AS, 100 & 200 kg N ha<sup>-1</sup>), and compost (200 kg N ha<sup>-1</sup>). The differential inhibitor, 1-octyne was used to distinguish AOA and AOB contributions to soil nitrification. Nine concentrations of ammonium (0-20 mM) were added to shaken soil slurries and nitrate accumulation from 2 to 24 h was determined. The Michaelis-Menten equation was used to fit nitrification rate to ammonium concentration. Maximum nitrification activity ( $V_{max}$ ) and half saturation constant ( $K_m$ ) of AOB ranged from 0.32 to 4.77 mM N kg<sup>-1</sup> d<sup>-1</sup> and 14 to 160  $\mu$ M NH<sub>4</sub><sup>+</sup> and both parameters were higher for soils that had received AS.  $V_{max}$  and  $K_m$  of AOA nitrification averaged 0.24 mM N kg<sup>-1</sup> d<sup>-1</sup> and 4  $\mu$ M NH<sub>4</sub><sup>+</sup> with no effect of N treatment. Soil slurries with 1 mM ammonium were also incubated at seven temperatures (5-50 °C). The Poisson density function equation was used to fit the nitrification rate to temperature. AOA fraction of nitrification potential was lowest at 5°C, increased with increasing temperature, and was near to 100% at 50°C. The optimum temperature was higher for AOA (41°C) than AOB (31°C). Understanding the niche differentiation of AOB and AOA as affected by fertilization may inform our ability to manage N more efficiently in agriculture.

[EN-16]

## The Effect of *Nitrobacter winogradskyi* and Heterotrophic Bacteria on the Proteome of *Nitrosomonas* sp. Is79

**Christopher Sedlacek**<sup>1</sup>, Ken Gries<sup>2</sup>, Wendy Haffey<sup>2</sup>, Tomislav Ticak<sup>1</sup> and Annette Bollmann<sup>1</sup>

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In the environment, microorganisms function within diverse communities and not as the often-studied pure cultures. We investigated the proteome of the ammonia-oxidizing bacterium *Nitrosomonas* sp. Is79 in continuous culture, in the presence of a nitrite oxidizer and co-cultivated heterotrophic bacteria using the enrichment culture G5-7 that contains: *Nitrosomonas* sp. Is79, *Nitrobacter winogradskyi*, and co-cultivated heterotrophic bacteria. The growth rate of *Nitrosomonas* sp. Is79 in batch culture increased when co-cultured with *N. winogradskyi* or heterotrophic bacteria. The proteome of *Nitrosomonas* sp. Is79 in co-culture with *N. winogradskyi* and heterotrophic bacteria was determined using an isobaric tag for relative and absolute quantification (iTRAQ) LC MS/MS approach. *Nitrosomonas* sp. Is79 displayed different proteomic changes when grown in co-culture with *N. winogradskyi* or as part of G5-7. The proteome of *Nitrosomonas* sp. Is79 had a decreased abundance of ATP synthase and cytoplasmic cellular oxidative stress response proteins when co-cultured with *N. winogradskyi*. In the enrichment culture G5-7, the changes in the proteome of *Nitrosomonas* sp. Is79 largely mirrored the changes observed when *Nitrosomonas* sp. Is79 was co-cultured with *N. winogradskyi*. In addition, the presence of heterotrophic bacteria resulted in an increased abundance of proteins involved in ammonia oxidation and a decreased abundance of proteins involved in amino acid synthesis. *Nitrosomonas* sp. Is79 is able to streamline its energy generation pathway and is protected from oxidative and nitrosative stress when co-cultured with *N. winogradskyi*. In addition, *Nitrosomonas* sp. Is79 appears to receive exogenous amino acids or amino acid metabolites when grown as part of G5-7.

[EN-17]

## **Influence of oxygen concentration and temperature on marine thaumarchaeal lipid composition confounds the TEX<sub>86</sub> paleotemperature proxy**

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Ammonia-oxidizing archaea (AOA) are among the most abundant microorganisms in the ocean. Their populations span diverse oceanic provinces and are now recognized to play a significant role in the nitrogen cycle. Modification of cell membrane composition in response to environmental change is thought to be important, in part, for their adaptive success and has also been used to infer oceanic conditions in the distant past. Specifically, the composition of their glycerol dibiphytanyl glycerol tetraether membrane lipids (GDGTs) are the basis for a widely used paleo sea surface temperature proxy (TEX<sub>86</sub>) that is applied to marine sediment records as far back as the Middle Jurassic. Despite the widespread assumption that temperature primarily regulates GDGT composition, several lines of evidence suggest that other factors could also play an important role in determining composition. However, until recently, no cultures were available for laboratory studies. Here we grow three marine AOA isolates under different growth temperatures and oxygen concentrations, and show that, in a single isolate, oxygen concentration is at least as important in controlling TEX<sub>86</sub> values as is temperature. The number of cyclopentane rings increased with increasing oxygen limitation, leading to significant elevations in TEX<sub>86</sub> derived temperature. Furthermore, we provide compelling evidence that strains SCM1, HCA1 and HCE1 have totally different TEX<sub>86</sub>-temperature relationships, revealing different temperature response characteristics among different presumptive ecotypes. Together our results challenge the generally accepted perception that GDGT cyclization in the ocean correlates solely with temperature. Although the adaptive significance of increased cyclization is unclear, this observation necessitates a reassessment of archaeal lipid-based paleotemperature proxies, particularly in records that span low oxygen events or underlie oxygen minimum zones.

[EN-18]

## The effect of organic carbon quality on nitrification and denitrification in existing stormwater biofilters

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Nitrogen excess is a key trigger for eutrophication of water bodies. Stormwater can be an important nitrogen source and requires effective treatment. However, the effectiveness of nitrogen removal is unsatisfactory because of the weakness of denitrification. A large-scale column study was conducted in Hefei, China, to test the performance of stormwater biofilters for the removal of nitrogen through the addition of special natural material in sandy loam, including Group A1 (terrestrial plant detritus), Group A2 (terrestrial plant detritus + manganese particles), Group A3 (terrestrial plant detritus + manganese particles + sludge), and Group B1 (aquatic plant detritus), Group B2 (aquatic plant detritus + manganese particles), Group B3 (aquatic plant + manganese particles + sludge) as well as control. The results demonstrated that plant detritus and sludge are critical to performance for nitrogen removal (e.g. aquatic plant detritus performed significantly better than terrestrial plant detritus), which was also proved by the higher nitrification and denitrification rate and higher abundance of ammonia oxidizing bacteria and Archaea in Group A3 and B3. This can be explained that plant detritus provided important organic carbon source for denitrification and sludge provided active nitrifying and denitrifying bacteria. Whilst phosphorus removal was consistently very high (typically around 80%), and manganese particles can markedly stimulate the phosphorus adsorption and ammonium and nitrate removal. Further trials will be required to test the bacterial community composition and possible mechanisms.

## **TOPIC 3: BIOTIC/ABIOTIC PROCESSES IN THE NITROGEN CYCLE**

### **POSTER & TALK**

**[BA-1] Monday Session, 5-6PM CCIS L1-140**

### **Effect of acid mine drainage on the abundance and diversity of freshwater nitrifying microbes**

**Annika Mosier**, Bhargavi Ramanathan, Joshua Sackett, Timberley Roane  
*University of Colorado, Denver*

Extremely acidic and metal-rich acid mine drainage (AMD) waters can have severe toxicological effects on aquatic ecosystems. AMD was shown to completely halt nitrification, which plays an important role in transferring nitrogen to higher organisms and in mitigating nitrogen pollution. We are evaluating whether AMD differentially impacts three groups of microorganisms involved in nitrification: ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB). Sediment and water were collected from AMD-impacted aquatic sites during June and August 2013 in the Iron Springs Mining District (Ophir, Colorado). Many of the sites were characterized by low pH (<5), low dissolved oxygen concentrations (<6 mg/L), and high metal concentrations. Community sequencing based on the 16S rRNA gene revealed the presence of AOA (*Nitrososphaera* and *Nitrosopumilus*), AOB (*Nitrosomonas*), and NOB (*Nitrospira*) at multiple AMD-impacted sites. The overall abundance of AOA, AOB and NOB were examined using quantitative PCR (qPCR) amplification of the *amoA* and *nxrB* functional genes. Bacterial *amoA* and *Nitrobacter nxrB* genes were not PCR amplifiable, though additional primer sets are still being evaluated. Archaeal *amoA* gene copy numbers ranged from 24-2.9x10<sup>4</sup> copies/μl of sediment DNA. *Nitrospira nxrB* gene copy numbers ranged from 80-3.5x10<sup>5</sup> copies/μl of sediment DNA. Overall gene abundance was somewhat correlated with dissolved copper concentrations for both groups (R<sup>2</sup>=0.6 for AOA *amoA* and R<sup>2</sup>=0.7 for *Nitrospira nxrB*). There were no significant correlation between gene abundance and other environmental parameters (e.g., pH, temperature, conductivity, dissolved oxygen, or other metals). These findings extend our understanding of the relationship between AMD and freshwater nitrifying microbes and provide a platform for further research.

## POSTER & TALK

[BA-2] Monday Session, 5-6PM CCIS L1-140

### **The role of surface nitrification in nitrous oxide production in mid-latitude North Atlantic**

**Qixing Ji** and Bess Ward

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The ocean is a major source of nitrous oxide, an important green house gas and ozone depleting agent. The majority of the world's ocean is well oxygenated and therefore nitrification is regarded as the major pathway of N<sub>2</sub>O production, because the other pathway, denitrification is inhibited by oxygen. To characterize the N<sub>2</sub>O production in oxygenated waters, <sup>15</sup>N tracer (<sup>15</sup>N-ammonium and <sup>15</sup>N-nitrite) incubations were used to measure directly the distribution of N<sub>2</sub>O production in mid-latitude North Atlantic waters. Because the surface N<sub>2</sub>O concentration is close to equilibrium with the atmosphere, N<sub>2</sub>O production in this region is generally regarded as insignificant. However active N<sub>2</sub>O production was detected from ammonium oxidation within the well-mixed surface layer. The peak of N<sub>2</sub>O production was vertically separated from peak ammonium oxidation, which occurred below the euphotic zone. The oxygen concentration minimum corresponded to peak N<sub>2</sub>O concentrations, where in situ N<sub>2</sub>O production was low. Nitrifier denitrification (production of <sup>15</sup>N-N<sub>2</sub>O from <sup>15</sup>NO<sub>2</sub><sup>-</sup>) was sporadically detected; its contribution to total N<sub>2</sub>O production was less significant than ammonium oxidation (<sup>15</sup>N-N<sub>2</sub>O from <sup>15</sup>NH<sub>4</sub><sup>+</sup>). The apparent N<sub>2</sub>O yield, i.e. the molar ratio of N<sub>2</sub>O-N production over nitrite production, was generally around 1% at the peak N<sub>2</sub>O production depths in the euphotic zone, and decreased to less than 0.01% at peak ammonium oxidation depths. The high N<sub>2</sub>O yield suggested the loss of fixed nitrogen during ammonium oxidation within the surface layer in mid-latitude North Atlantic. However the N<sub>2</sub>O flux from this region is miniscule compared to total oceanic flux.

[BA-3] *also a workshop presentation*

## **Isotopic and Kinetic Investigations of Abiotic Nitrous Oxide Formation from Nitrification Intermediates and Redox-Active Metals in Seawater**

**Jennifer B. Glass**<sup>1</sup>, Amanda R. Cavazos<sup>1</sup>, Chloe L. Stanton<sup>1</sup>, Martial Taillefert<sup>1</sup>, Joshua A. Haslun<sup>2</sup>, Nathaniel E. Ostrom<sup>2</sup>

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Nitrification contributes a significant source of N<sub>2</sub>O in marine ecosystems, which comprise approximately one third of global N<sub>2</sub>O sources. Previous studies have carried the implicit assumption that marine N<sub>2</sub>O originates directly from enzymatic reactions. However, abiotic production of N<sub>2</sub>O is possible via chemical reactions between nitrification intermediates and redox active trace metals in seawater. Thermodynamic calculations predict that NH<sub>2</sub>OH oxidation to N<sub>2</sub>O will occur at pH 7-8 with both Mn(IV) and Fe(III) oxides. Abiotic NO reduction with Fe(II) to N<sub>2</sub>O is also thermodynamically favorable at circumneutral pH. In this study, we investigated the rates and isotopic signatures of abiotic N<sub>2</sub>O production from NH<sub>2</sub>OH and NO in artificial seawater under oxic and anoxic conditions, respectively. Kinetic studies with microelectrodes showed that rates of N<sub>2</sub>O production via NH<sub>2</sub>OH oxidation were significantly faster and exhibited greater yield with Mn(IV) vs. Fe(III), and that reaction rates increased with pH. Isotopomer site preference (SP) of N<sub>2</sub>O produced from abiotic NH<sub>2</sub>OH oxidation was consistent with previous studies (30 ± 4 ‰). The SP of N<sub>2</sub>O produced from abiotic NO reduction (16 ± 3 ‰) was significantly different than that of N<sub>2</sub>O from NH<sub>2</sub>OH oxidation or biological denitrification. This study suggests that coupled biotic-abiotic N<sub>2</sub>O production may occur in marine environments where nitrification takes place in the presence of elevated Fe, Mn and/or other redox-active metals, such as coastal areas, oxygen minimum zones, and near the sediment-water interface, and that abiotic N<sub>2</sub>O production from NO may carry an isotopic site preference that is distinct from other biological and abiotic production pathways.

[BA-4]

## **N<sub>2</sub>O Dynamics in Restored Peatlands**

**Martin E. Brummell**, Maria Strack  
*University of Waterloo, Waterloo, Ontario, Canada*

While peatland ecosystems, in common with other wetlands, are known to be important components of carbon-based greenhouse gas exchange, the movements, transformations, and release of N<sub>2</sub>O from such ecosystems is poorly understood. Denitrification is both an important source of N<sub>2</sub>O and the only known biological sink, through the action of nosZ during the final step of complete denitrification. Nitrifying and denitrifying organisms are abundant in peatlands, and their populations respond to the changes in hydrology and vegetation associated with harvesting peatlands and subsequent restoration efforts. Net emissions of N<sub>2</sub>O will be measured by a gas chromatograph at the University of Waterloo from samples collected from chambers in a peatland near Seba Beach, Alberta over the growing season of 2015. Soil, water, and vegetation samples will be collected from pits excavated within the restored and undisturbed areas of the site. Microbial DNA extracted from soil samples will be analyzed by qPCR for N-cycle genes including nirS, nirK, nosZ, and amoA to measure relative abundance of nitrifying and denitrifying organisms. Potential nitrification and denitrification will be measured in laboratory microcosms using soils collected and frozen in the field. Net N<sub>2</sub>O emissions, potential nitrification and denitrification rates, and the distributions of populations of nitrifying and denitrifying organisms will provide an overview of the movements and transformation of nitrogen compounds in this ecosystem, thus providing a foundation for further studies of the factors, both biotic and abiotic driving greenhouse gas emissions.

[BA-5]

## Cyanate as an alternative substrate for nitrifiers in terrestrial ecosystems

**Maria Mooshammer**<sup>†</sup>, Marton Palatinszky<sup>†</sup>, Craig Herbold<sup>†</sup>, Ping Han<sup>†</sup>, Holger Daims<sup>†</sup>, Andreas Richter<sup>‡</sup>, and Michael Wagner<sup>†</sup>

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For decades, urea and ammonia have been considered as the only two compounds that can serve as sole source of energy and reductant during aerobic growth of ammonia-oxidizing bacteria and archaea. However, we could recently show that a pure culture of the ammonia-oxidizing thaumarchaeote *Nitrososphaera gargensis* grows aerobically on cyanate (OCN<sup>-</sup>) as only energy and nitrogen source (Palatinszky et al., 2015). *N. gargensis* and all known nitrite-oxidizers possess a cyanase, which catalyses the hydrolysis of cyanate to ammonium and carbon dioxide. We also demonstrated that reciprocal feeding between nitrite-oxidizers and cyanase-deficient ammonia oxidizers enables growth of both partners on cyanate. Furthermore, published metagenomes revealed that cyanase-encoding genes closely related to those of nitrifiers are widespread in the environment.

Surprisingly, cyanate concentrations and fluxes in natural environments are largely unknown, and environmental cyanate concentrations have only been studied in seawater so far, where it was found to occur in the nanomolar-range (Widner et al. 2013). No information about the importance of cyanate in soils is available, although urea that spontaneously decomposes to cyanate is on a global scale the most used agricultural fertilizer. Cyanate can have many fates in soils - it can be (1) used as nitrogen and/or energy source by cyanase-encoding microorganisms, (2) abiotically hydrolysed to ammonium and carbon dioxide, (3) adsorbed to soil particles, or (4) complexed with other compounds. Here we present the first results of experiments designed to differentiate between biotic and abiotic degradation of cyanate in soils and introduce new techniques that should allow us to study the importance of nitrifier cyanate metabolism in terrestrial ecosystems.

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[BA-6]

## **Biotransformation of pharmaceuticals by ammonia-oxidizing archaea and bacteria**

**Ping Han<sup>1</sup>**, Yujie Men<sup>2</sup>, Michael Wagner<sup>1</sup>, Kathrin Fenner<sup>2</sup>

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Micropollutants, including pharmaceuticals, personal care products, industrial chemicals and many other emerging compounds are an increasing worldwide contamination of freshwater systems at trace concentrations yet raising considerable toxicological concerns. In recent studies, the activity of ammonia oxidizing microbes was reported to be strongly correlated with biotransformation of a variety of these micropollutants (Helbling et al., 2012; Khunjar, et al., 2011; Tran et al., 2013). To explore more directly the roles of ammonia oxidizers in biotransformation of micropollutants, eleven selected micropollutants (40 µg/L each) representing four different categories were added as a mixture together with 2mM ammonium chloride to pure cultures of the ammonia-oxidizing thaumarchaeote *Nitrososphaera gargensis* and of two *Nitrosomonas* strains, respectively. Two pharmaceuticals - mianserin and ranitidine - were up to 80% and 60 %, respectively, degraded after five days of incubation by all three strains. Further experiments using 100 µg/L of these pharmaceuticals confirmed that they were individually biotransformed by all tested ammonia oxidizers along with ammonium consumption, indicating a co-metabolic mechanism. Interestingly, *N. gargensis* showed faster biotransformation rates of mianserin and ranitidine than the ammonia-oxidizing bacteria. Downstream analysis confirmed 1-oxo mianserin as the major transformation product of mianserin, while the transformation products of ranitidine still need to be investigated. In the next step, we will compare micropollutant biotransformation in nitrifying activated sludge after addition of selective inhibitors of ammonia-oxidizing archaea and bacteria, respectively, to obtain insights into the specific contribution of these two clades to this process in a complex ecosystem.

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## **TOPIC 4: DIVERSITY & MODULARITY IN THE NITROGEN CYCLE**

### **POSTER & TALK**

**[DM-1] Monday Session, 5-6PM CCIS L1-140**

### **Isolation and successful subculture of *Nitrosomonas mobilis* lineage: Recovery of nature's lost treasure since 1970's**

**Hirotsugu Fujitani**, Asami Kumagai, Norisuke Ushiki, Kengo Momiuchi, and Satoshi Tsuneda

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Nitrification consists of two step reactions, ammonia oxidation by ammonia-oxidizing bacteria (AOB) or archaea (AOA) and nitrite oxidation by nitrite-oxidizing bacteria (NOB). Although *Nitrosomonas mobilis* lineage is a representative AOB group and a few pure strains of this lineage were isolated, approaches to preserve these strains in pure culture have not been established. Therefore, their physiological and biochemical characteristics remain unknown for some decades. In our previous study, we developed an isolation method focusing on microcolonies formation of nitrifying bacteria and obtained novel strains in the phyla of Nitrospira, which is most important NOB in wastewater treatment processes. Actually, inoculation of a pure microcolony, not a single cell as a growth unit, led to sub-culture successfully and increased probability of isolation of uncultured NOB. We conceived this method to be applicable for other nitrifying bacteria, such as AOB capable of microcolony formation. Here, we report novel isolates belonging to *N. mobilis* lineage from autotrophic nitrifying granules used for ammonia-rich wastewater treatment. Obtained pure strains could be preserved in inorganic medium containing ammonia under low temperature and recovered successfully. Strain Ms1 is potentially cultivated in the liquid culture with relatively high ammonia or nitrite concentration, not extremely slow growing, which agreed with the findings of Koops et al. Considering environmental clones closely related to *N. mobilis* that were detected in activated sludge, recirculating aquaculture systems, wetland and so on, strain Ms1 could be also a standard strain due to the ease in handling to reveal this member's ecophysiology in habitats.

[DM-2] *also a workshop presentation*

## **Hydrogen peroxide detoxification by $\alpha$ -keto acid oxidation is required for stimulation of growth of a marine ammonia-oxidizing archaeon**

**Jong-Geol Kim**, Ok-Ja Si, Sung-Keun Rhee

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Thaumarchaeota are abundant in marine environments and are known to be involved in ammonia oxidation. Potential of organic carbon assimilation by AOA is still under debate. We isolated an ammonia-oxidizing archaeon (designated strain DDS1) from seawater which requires supplementation of organic acids in medium for its growth. Unexpectedly, a tracer experiment indicates that organic carbon incorporation into archaeal cellular lipids was negligible and most of lipids carbons are from dissolved inorganic carbons. Further, any scavenger of hydrogen peroxide ( $H_2O_2$ ) such as dimethylthiourea and catalase could replace organic acid requirement. In fact, all organic acids stimulated the growth of strain DDS1 were  $\alpha$ -keto acids which can degrade  $H_2O_2$  by nonenzymatic decarboxylation. This was verified by that only carboxyl carbon of pyruvate carbons was released into medium. This result indicates that strain DDS1 is a strict autotrophic archaeon. Genomic analysis indicates that all known AOA strains including strain DDS1 are lack of putative catalase genes and might be sensitive to high concentration of  $H_2O_2$ . Indeed, strain DDS1 was shown to endogenously produce  $H_2O_2$  (upto ca.  $0.3 \mu M$ ) in the absence of  $\alpha$ -keto acid which was inhibitory to the growth of strain DDS1. Growth of strain DDS1 in the absence of  $\alpha$ -keto acid could be enhanced by coupling with catalase-positive heterotrophs, indicating  $H_2O_2$  degradation by extant bacteria are associated with archaeal ammonia oxidation. Our results indicates that  $H_2O_2$  is implicated as a key factor determining activity and distribution of AOA ecotypes and thus affecting biogeochemical cycles of nitrogen and carbon in marine environments.

**[DM-3] also a workshop presentation**

## **Impact of hydroxylamine on the growth kinetics and gene expression of enriched *Nitrospira* spp.**

**Mee-Rye Park** and Kartik Chandran

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The next generation of energy and resource efficient engineered biological nitrogen removal (BNR) processes is based on the foundation of selective suppression of nitrite oxidizing bacteria (NOB) related specifically to *Nitrospira* spp. in dilute nitrogen containing streams (similar to domestic sewage). The overarching goal of this study is to determine the impact of nitrogenous intermediates in the overall microbial N-cycle (such as hydroxylamine, presented herein) on the growth kinetics and gene expression profiles of *Nitrospira* spp.

Cultures of *Nitrospira* spp. were successfully enriched from a real wastewater treatment plant and exposed to hydroxylamine concentrations in the range 1 - 5 mg-N/L. These concentrations were selected, having previously been measured in engineered nitrification processes. Hydroxylamine exposure resulted in significant inhibition of nitrite oxidation rates (measured using respirometric assays) with a non-competitive inhibition coefficient (corresponding to 50% inhibition relative to control) of 4.2 mg-N/L. In parallel, expression of genes coding for catabolism (nitrite oxidation, *nxB*) and anabolism (carbonic anhydrase, *cah* and CO<sub>2</sub> fixation, *porA* and *forA*) all also decreased upon hydroxylamine exposure. In addition, an up-regulation of stress response genes (*btuE* and *oxyR*) during exposure to hydroxylamine was observed.

These results demonstrate that intermediates such as hydroxylamine can strongly inhibit the activity and expression of key anabolic and catabolic pathways of *Nitrospira* spp.. Therefore, strategies that rely upon the transient accumulation and consumption of such intermediates (such as transient aeration) could provide the platform for successful suppression of *Nitrospira* spp. in the next generation of energy efficient engineered BNR processes.

[DM-4]

## **A chemolithoheterotrophic ammonia-oxidizing archaeon *Nitrosopumilus* sp. NM25 isolated from eelgrass zone sediment**

**Tatsunori Nakagawa** 1,2, Mori Koji 3, Reiji Takahashi 1, and David A. Stahl 2  
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Marine ammonia-oxidizing archaea belonging to the phylum Thaumarchaeota distribute globally in sea sediments, besides being key organisms in the global nitrogen cycle. The morphological and physiological properties of an organism isolated from the eelgrass zone sediment, provisionally called *Nitrosopumilus* sp. NM25, were investigated. A highly enriched strain NM25 culture was maintained for two years by using the successive transfer of 10% subcultures into the same 15 mM ammonium-containing medium buffered with MOPS to pH 8.0. After changing the medium to the 1 mM ammonium-containing medium buffered with HEPES to pH 7.5, supplementation of streptomycin, filtration of cultures, and serial dilution of the cultures were conducted to isolate the strain NM25. Electron microscopy indicated that the cells were nonmotile rods (diameter, 0.20-0.36  $\mu\text{m}$ ; length, 0.35-0.59  $\mu\text{m}$ ) and that the cells surface were consist of an S-layer protein. Strain NM25 produced 1 mM nitrite for 13 days in the 1 mM ammonium-containing medium with 0.5 mM bicarbonate and 0.1 mM alpha-ketoglutarate, but produced only 0.2 mM nitrite in the absence of alpha-ketoglutarate. When 0.1 mM alpha-ketoglutarate was added to the culture after 14-day of incubation without alpha-ketoglutarate, the concentration of nitrite in the culture increased again up to 1 mM. These data suggest that strain NM25 is a chemolithoheterotrophic ammonia-oxidizing archaeon, and that it may play a role in organic carbon transformations in marine sediments.

[DM-5]

**An obligatorily autotrophic ammonia-oxidizing archaeon, “*Candidatus Nitrosocosmicus oleophilus*”, affiliated to thaumarchaeotal group I.1b isolated from a coal tar-contaminated soil**

**Man-Young Jung**, Heeji Hong, Sung-Keun Rhee  
*Department of Microbiology, Chungbuk National University*

Nitrification is an essential component of the global nitrogen cycle for agricultural productivity and is involved in atmospheric and groundwater pollution. Members of I.1b groups of Thaumarchaeota are considered to harbor ammonia-oxidizing microorganisms especially dominant in various soils. In this study, we isolated an ammonia-oxidizing archaeon designated strain MY3 that belongs to a “fosmid clone 29i4 clade” of thaumarchaeal group I.1b, which have few cultivated representatives. Strain MY3 was isolated from a coal tar-contaminated soil. Strain MY3 was observed to be mesophilic (optimum temperature being 30°C), and neutrophilic (optimum pH 7 to 7.5). Growth assays showed that supplementation of various organic carbons had no effect on the ammonia-oxidation rate or growth yields of strain MY3, which indicates that strain MY3 is strictly chemolithoautotrophic. In addition, a <sup>13</sup>C-bicarbonate-assimilation assay showed stoichiometric incorporation of <sup>13</sup>C into archaeal cellular lipids. Cells of strain MY3 were aggregated each other and formed clusters. Ammonia-oxidizing activity of strain MY3 was stimulated by supplementation of hydrophobic materials. Most of strain MY3 cells could be extracted using hydrophobic solvents. Genome analysis of strain MY3 showed that genes related to surface adhesion and aggregation were enriched. On the basis of phenotypic, phylogenetic, and genomics characteristics, we propose the name “*Candidatus Nitrosocosmicus oleophilus*” for the ammonia-oxidizing archaeal strain MY3.

[DM-6]

## Identification of acyl-homoserine lactone autoinducers produced by the nitrite-oxidizing bacterium *Nitrobacter winogradskyi*

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*Nitrobacter winogradskyi* is a chemolithotrophic, nitrite-oxidizing bacterium that plays a role in the nitrogen cycle. Here, we demonstrate a functional N-acyl-homoserine lactone (acyl-HSL) synthase in this bacterium. The *N. winogradskyi* genome contains genes encoding an acyl-HSL autoinducer synthase (Nwi\_0626, *nwiI*) and an acyl-HSL autoinducer receptor (Nwi\_0627, *nwiR*) with amino acid sequences similar to those in *Rhodopseudomonas palustris* and other Rhizobiales. Expression of *nwiI* and *nwiR* correlated with acyl-HSL production during culture. *N. winogradskyi* produces two distinct acyl-HSLs, N-decanoyl-L-homoserine lactone (C10-HSL) and a monounsaturated acyl-HSL (C10:1-HSL), in a cell-density- and growth-rate-dependent manner, during batch and chemostat culture. The acyl-HSLs were detected by bioassay and identified via ultra-performance liquid chromatography, information-dependent acquisition, mass spectrometry (UPLC-IDA-MS). The C=C bond in C10:1-HSL was confirmed by conversion into bromohydrin and detection by UPLC-IDA-MS. To our knowledge, this is the first demonstration of acyl-HSL production by a nitrite-oxidizing bacterium. Acyl-HSL quorum sensing may be one method of inter-species cell-cell signaling between ammonia oxidizers and nitrite oxidizers, and may contribute to the efficient coupling of ammonia and nitrite oxidation during nitrification.

[DM-7] *also a session presentation*

## **The chemistry of biological ammonia-nitrite interconversion: Insights from studies of cytochrome c nitrite reductase**

**A. Andrew Pacheco**

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Cytochrome c Nitrite Reductase (ccNiR, or NrfA) is a periplasmic, decaheme homodimeric enzyme that catalyzes the six-electron reduction of nitrite to ammonia (ammonification). Under physiological conditions ccNiR catalyzes the process without release of intermediates. However, in vitro it is possible to trap putative intermediates, and even to force the enzyme to carry out part of its reaction sequence in reverse. Such experiments provide possible insights not only about ccNiR and ammonification, but also about nitrification and the enzymes that catalyze this process. UV/Visible stopped-flow and electron paramagnetic resonance freeze-quench studies of the interaction between hydroxylamine and ccNiR under anaerobic conditions showed that hydroxylamine can stoichiometrically reduce ccNiR, generating free nitrite and ccNiR-bound nitrogen intermediates. Importantly ccNiR did not catalyze the disproportionation of hydroxylamine to give ammonia and nitrite, despite the fact that the process is very favorable thermodynamically, a result that implicated hydroxylamine reduction as the rate limiting step in ccNiR-catalyzed ammonification. Controlled potential electrolysis studies of ccNiR in combination with UV/visible absorption and electron paramagnetic resonance spectroscopies were consistent with this hypothesis. When electrolysis was performed with the active site fully nitrite-loaded, the first step was indisputably a concerted 2-electron reduction (occurring at 20 mV vs SHE) that produced a {Fe(NO)}<sup>7</sup> species at the active site. Only by decreasing the applied potential below about -120 mV vs SHE could further reduction of {Fe(NO)}<sup>7</sup>, presumably to release ammonia, take place. We propose that the low applied potential is required to overcome the kinetic barrier to the final 2-electron reduction.

[DM-8]

## **Selective enrichment of uncultured ammonia-oxidizing bacteria and archaea and *Nitrospira* from freshwater by continuous feeding bioreactors**

**Kengo Momiuchi** \*, Hirotsugu Fujitani \*, Satoshi Tsuneda \*

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Cultivation of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) and *Nitrospira* are very difficult compared with that of other heterotrophic bacteria. The reason includes growth inhibition of AOA and AOB and *Nitrospira* with a high concentration of substrate such as ammonia and nitrite. Therefore, keeping low ammonia concentration is required to enrich nitrifiers from fresh water. In this study, we developed continuous feeding bioreactors for selective enrichment of AOA and AOB by controlling their substrate concentration. Bioreactor I was provided with inlet ammonia concentration of 1 - 60 mg-N L<sup>-1</sup> and bioreactor II with 1 mg-N L<sup>-1</sup> as well for one year. The biological activated carbons from a freshwater river were used as primary inoculums. The ratio of nitrifying microorganisms to the total microorganisms was measured by fluorescence in situ hybridization direct counting. In bioreactor I, the AOB affiliated with *Nitrosomonas oligotropha* lineage were enriched selectively by maintaining ammonia concentration within 0.1 mg-N L<sup>-1</sup>, while the AOA with Soil group I.1b were cultivated in bioreactor II in which the ammonia was completely consumed. In 350 days, the maximum ratios of AOB and *Nitrospira* to total microbial cells in bioreactor I achieved 33.4% and 42.6%, respectively. The ratio of AOA and *Nitrospira* in bioreactor II increased to 24.3% and 15.1%, respectively. These results demonstrated that selective enrichment of AOA and AOB is possible, considering remarkable difference in substrate affinity between AOA and AOB.

[DM-9]

## **Multi-color DOPE-FISH – a method to enable detection, visualization and quantification of *Nitrospira* microdiversity, colocalization and interactions in waste water treatment plants and beyond**

**Petra Pjevac**, Adrian Berger, Markus Schmid, Christiane Gruber-Dorninger, Michael Wagner, Holger Daims

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Nitrite oxidation in wastewater treatment plants (WWTPs) is usually dominated by uncultured *Nitrospira*. Monophyletic, closely related clusters from *Nitrospira* phylogenetic lineages I and II were shown to frequently co-occur, while exhibiting in situ population shifts and abundance fluctuations. Preceding studies showed that co-existence of closely related *Nitrospira* is facilitated through niche partitioning based on substrate level preferences, oxygen tolerance and carbon source range. Various methods (e.g MAR-FISH, Raman-FISH or NanoSIMS) enable a functional analysis of these microdiverse *Nitrospira* assemblages. However, the absence of a robust rRNA targeting FISH technique, which allows the simultaneous in situ detection of all co-occurring *Nitrospira* populations, complicates data generation and interpretation.

Recently, significant efforts have been invested in methods development to extend the number of target organisms detectable in a single rRNA-FISH assay. However, available methods cause biases including decreased sensitivity and objectionable probe binding. To circumvent these, we established a FISH approach exploiting the broad wavelength spectra available through latest generation confocal laser scanning microscopy and fluorophores with unusual excitation and fluorescence spectra. The use of 5 new fluorophores allows the simultaneous detection of 8 target populations by single-labeled oligonucleotides. Furthermore, the prospect of a simultaneous detection of up to 36 target populations by combining alternative fluorophores with double labeling of oligonucleotide probes FISH (DOPE-FISH) is currently under development. This extended multi-color DOPE-FISH method holds the potential to tremendously advance and simplify analyses of microdiversity, colocalization and interactions of *Nitrospira* and other microorganisms, not only in WWTPs, but also in various environmental samples.

[DM-10]

## Alternative roles of nitrite-oxidizing bacteria in- and outside the nitrogen cycle

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Nitrification, the stepwise oxidation of ammonia to nitrate via nitrite, represents a tight interplay between ammonia-oxidizing microbes (AOM) and nitrite oxidizing bacteria (NOB), which gain their energy by the oxidation of nitrite. For decades NOB were considered to be highly dependent on the supply of nitrite by AOM and nitrate-reducers. In this study the genome analysis of *Nitrospira moscoviensis*, member of the widely distributed *Nitrospira* lineage II, was basis for investigating the metabolic flexibility of this clade of NOB. The genetic repertoire of *N. moscoviensis* includes the machineries for hydrogen and formate oxidation and urea utilization. Incubation experiments confirmed the ureolytic activity of this nitrite oxidizer. Additionally, the ureolytic *Nitrospira* cleaved urea to ammonia to fuel nitrification in co-incubations with the non-ureolytic AOB *Nitrosomonas europaea*. Interestingly, the potential to use urea seems to be not restricted to this single *Nitrospira* species. A similar urease gene cluster was found in the genome of *Nitrospira lenta* from activated sludge. The observed ureolysis of NOB for starting nitrification is a novel and unexpected aspect in the symbiosis of AOM and NOB. In addition, the usage of alternative energy sources of *Nitrospira* challenges the classical assumption of their dependency on AOM. Recently, we showed that *N. moscoviensis* can use hydrogen as sole energy source for chemolithoautotrophic CO<sub>2</sub> fixation and grows aerobically on hydrogen without nitrite. Furthermore, anaerobic formate oxidation coupled to nitrate reduction allows *Nitrospira* to participate in reductive nitrogen cycle pathways. Taken together, our results elucidate an unknown metabolic flexibility of *Nitrospira*.

[DM-11]

## **Assessing and optimizing methanotroph growth conditions for use in industrial applications**

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Methane gas, a pollutant significantly contributing to the greenhouse gas effect, is a common by-product or waste from industrial activities. While its release and sequestration is a major concern to both governments and private industries, methane can also serve as a useful and economical feedstock for methanotrophs, which have long been noted for their immense potential in the field of biotechnology, including for the biosynthesis of desirable biomaterials and metabolites. A particular bioproduct of interest, native to methanotrophs, is polyhydroxybutyrate (PHB), a precursor to next generation bioplastics. However, optimization of bioproduction relies upon understanding the growth and behaviour of methanotroph species as they relate to growth substrates such as nitrogen source. This understanding can be achieved by characterizing and synthesizing growth of cultures and the cellular regulation of metabolic pathways, including those involved in producing PHB. To accomplish this, global gene expression analysis along bacterial growth under varying growth conditions was performed, to highlight differences in expression induced by substrate choice. This approach is vital to optimizing cellular biomass and PHB production and achieving a cost-efficient means of production and may also lead to the identification of other metabolites with economic relevance. The potential revealed through this project could result in production of materials that are useful to society, non-harmful to the environment, and profitable for both bioplastic producers and industries releasing methane as a by-product.

[DM-12]

## **Phosphorus utilization strategy of nitrogen-fixing cyanobacteria----*Anabaena flos-aquae***

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Cyanobacterial bloom events threaten the health of ecosystems. *Anabaena flos-aquae* is one of the bloom-forming species in Chinese large shallow lakes. *Anabaena flos-aquae* can fix nitrogen to overcome its limitation in freshwater, while their strategy to use phosphorus and the role of nitrogen fixation during the process remain unclear. Widely in situ investigation and laboratory experiment showed that *Anabaena* can effectively use dissolved organic phosphorus by excreting extracellular phosphatase as evidence by the positive enzymatic labeling of fluorescence (ELF), further molecular study found *PhoD*, the gene encoding phosphatase (the enzyme involved in the hydrolysis of phosphomonoesters) in the isolation. The location of the ELF labeling around the heterocyst, where nitrogen fixation takes place, indicated the close relationship between nitrogen and phosphorus utilization.

## TOPIC 5: ENGINEERED ENVIRONMENTS

POSTER & TALK: Tuesday Session, 5-6PM, CCIS L1-140

[EE-1]

### Taxonomic and metagenomic profiling of rapid sand filter microbiome reveals a high *Nitrospira* incidence

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Rapid sand filtration is a drinking water production technology, widely used in Europe and around the world, for the removal of ammonia, reduced manganese and iron, methane, hydrogen sulfide and other compounds from groundwater by a combination of physical, chemical and biological processes. Microbially catalyzed processes dominate the oxidative transformations of most contaminants. Our interest is to elucidate the link between the microbial composition in these rapid sand filters (RSFs) and the removal of contaminants. In this study, six samples from a RSF were analyzed through complementary 16S rRNA tag pyrosequencing and metagenomic approaches. 16S rRNA based 454 sequencing revealed that Nitrospirae, Proteobacteria and Acidobacteria dominated in all samples (89.56%±19). Gemmatimonadetes and Candidate division OD1 constituted secondary major clades in the investigated filters. Higher resolution analysis showed a highly diverse sub-genus phylogeny in the *Nitrospira* clade with some OTUs of the previously defined lineage I (*Nitrospira defluvii*) and lineage II (*Nitrospira moscoviensis*), but mostly of previously uncharacterized *Nitrospira* lineages. The community gene catalogue retrieved most genes of the nitrogen cycle, with special abundance of genes in the nitrification pathway. Genes involved in different CO<sub>2</sub> fixation pathways were also present, with the rTCA pathway the most abundant, consistent with the *Nitrospira* dominance observed in the 16S rRNA libraries. From the metagenomic data set, near-complete genomes were reconstructed and functionally characterized. Based on the genetic content, a Methylococcaceae related genome showed the capacity of methane oxidation whereas one Rhizobiales genome is putatively involved in manganese oxidation. Several reconstructed genomes had the potential for organic carbon compound degradation. Several distinct genomes of the *Nitrospira* genus were recovered indicating microdiversity within this taxon. The unexpected abundance and recently described metabolic versatility may indicate a major role of *Nitrospira* lineage in investigated RSFs. This investigation has so far revealed the potential actors and main biological processes occurring in the RSF environment. In the next step, metatranscriptomic analysis will be performed to elucidate the physiological activity of *Nitrospira* spp, the interdependencies between the dominant actors, and their link with the biogeochemical transformations.

POSTER & TALK: Tuesday Session, 5-6PM, CCIS L1-140

[EE-2]

## **Influence of Wastewater Constituents on the Toxicity of Silver Nanoparticles to the Model Ammonia Oxidizing Bacterium, *Nitrosomonas europaea***

Cameron Kostigen Mumper<sup>1</sup>, Ann-Kathrin Ostermeyer<sup>1</sup>, Leila Barker<sup>2</sup>, Joseph Anderson<sup>2</sup>, Lewis Semprini<sup>2</sup> and **Tyler S. Radniecki**<sup>1,2</sup>

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The rapid and unregulated introduction of silver nanoparticles (AgNPs) to the consumer market has raised concerns over their potential to impair essential biological processes within wastewater treatment plants. This work examines the toxicity AgNPs, of various sizes and shapes, to *Nitrosomonas europaea*, a model ammonia oxidizing bacteria found in WWTPs, in the presence and absence of surrogate wastewater and surface water constituents. Additionally, this work examines how the physiological growth state of *N. europaea* influences their sensitivity to AgNPs. The toxicity of the AgNPs was determined to be due to the release of Ag<sup>+</sup>, which increased in the presence of NH<sub>3</sub>, due to the formation of Ag(NH<sub>3</sub>)<sub>2</sub><sup>+</sup>. Dissolution rates decreased with increasing AgNP size, however triangular AgNPs had much higher dissolution rates than equal-sized spherical AgNPs. AgNP toxicity was reduced when dissolution rates were decreased and/or the released Ag<sup>+</sup> were sequestered by other aqueous constituents. Divalent cations induced AgNP aggregation, which decreased dissolution rates, and prevented Ag<sup>+</sup> from binding to *N. europaea* cells. Proteins, polysaccharides and humic acids coated the AgNPs, preventing NH<sub>3</sub>-dependent dissolution and the adsorption of AgNPs onto the cells. However, only proteins were able to sequester released Ag<sup>+</sup> through Ag<sup>+</sup>-thiol interactions. Biofilms were found to be more resilient to AgNP inhibition due to decreased growth rates and diffusion gradients through the thickness of the biofilm.

POSTER & TALK: Tuesday Session, 5-6PM, CCIS L1-140

[EE-3]

## Physiological and proteomic responses of methane and ammonia cometabolism in *Nitrosomonas europaea*

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Our research, which is motivated by the well-established structural and functional homology between ammonia monooxygenase and particulate methane monooxygenase, examines the cometabolism of methane and nitrogen by ammonia oxidizing bacteria at cellular level. Results from this study hold utility to assess the impact of dissolved methane in shallow groundwater (e.g., resulting from hydraulic fracturing operations) on nitrogen cycling in natural systems. In engineered systems, our research lays the foundation for biogenic methane to methanol production to enhance the sustainability of water resource recovery facilities. Here, we present results from experiments where *Nitrosomonas europaea* in a chemostat culture ( $D = 0.5 \text{ d}^{-1}$ ) fed with ammonia (feed concentration =  $280 \text{ mgNL}^{-1}$ ) were exposed to methane gas pulse input for ~3 days. Results showed that methane exposure resulted in rapid washout of biomass, with the effluent ammonia concentration increasing to maximum of  $200 \text{ mgNL}^{-1}$ . Methanol peaked within 1-day of methane exposure at a concentration of  $25 \text{ mgCODL}^{-1}$  and subsequently decreased to non-detect levels. The system returned to steady-state 14 days after the methane was stopped. Proteomic profiles revealed that of the 355 identified proteins, 166 proteins were present in all operating phases, while 3, 22, and 98 proteins were exclusively identified in pre-exposure, methane exposure, and post exposure phase, respectively. Preliminary analysis showed that for those proteins uniquely identified during methane exposure, 27% (6/22) is related to heme biosynthesis. Taken together, results showed that although system level impacts may be reversible, distinct proteomic profile was observed after recovering from methane exposure.

[EE-4] also a workshop presentation

## Reactivation of microbial nitrogen cycling conversions after Lower Earth Orbit Space exposure

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Various processes within the microbial nitrogen cycle are considered as resource efficient alternatives to the physicochemical methods for recovery of both nitrogen and water for long-term manned Space missions. One of the major application challenges is to start up biological reactors with inocula that can be preserved under the conditions of microgravity and radiation conditions prevalent in Space. Furthermore, when a biological treatment system fails, re-inoculation should prevent that it take months to recover steady-state operation. In the current study, a Space flight was performed with (i) three natural microbial communities, containing members of the ammonia oxidizing archaea (AOA) and bacteria (AOB), nitrite oxidizing bacteria (NOB), denitrifiers and anammox bacteria (AnAOB), and with (ii) a synthetic culture of the ureolytic *Cupriavidus pinatubonensis*, the AOB *Nitrosomonas europaea* and the NOB *Nitrobacter winogradskyi*. The cultures were sent on a PHOTON-M4 flight to Lower Earth Orbit (LEO) Space and were exposed to  $20 \pm 4^\circ\text{C}$ , hyper and  $\mu$ -gravity and to  $30.5 \pm 6.9$  mGy of radiation over 44 days. Upon return to Earth the cultures were reactivated and volumetric activity in mg N L<sup>-1</sup> d<sup>-1</sup> was compared to the same cultures that were stored terrestrially at ambient temperature ( $23 \pm 3^\circ\text{C}$ ) and in the refrigerator ( $4^\circ\text{C}$ ). It should be noted that the measured background radiation on Earth was only  $1.6 \pm 0.1$  mGy over the same period. Nevertheless the LEO-samples performed either similar or better after reactivation compared to the ambient terrestrial stored cultures. Both the LEO and the ambient terrestrial stored cultures showed a significant decline in activity compared  $4^\circ\text{C}$  storage. More in-depth data on specific conversion rates, changes in biomass concentrations, cell viability tests using flow cytometry, Illumina-sequencing of the microbial communities is being processed. In conclusion, this study for the first time reports on the specific Space-flight survival capacity of the key conversions in the microbial nitrogen cycle, a necessary step in advancing toward a bio-regenerative life support system.

[EE-5] *also a workshop presentation*

## **Modeling the population dynamics during nitrification of urine**

**Alexandra Fumasoli**<sup>1</sup>, Eberhard Morgenroth<sup>1,2</sup>, Kai M. Udert<sup>1</sup>

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Microbial nitrification is a possible pretreatment step for fertilizer production from human urine. As the alkalinity to ammonia ratio in urine is low (about 1 mol alkalinity per 1 mol of ammonium), the pH can vary strongly depending on the inflow rate. This can lead to two major failures: the accumulation of the intermediate nitrite caused by the inhibition of nitrite oxidizing bacteria (NOB) and the selection of acid-tolerant ammonia oxidizing bacteria (AOB). The aim of the study was to set up a mathematical model explaining the previously observed population dynamics of acid-sensitive (*Nitrosomonas eutropha*) and acid-tolerant AOB (genus of *Nitrosococcus*) as well as their interplay with NOB (genus of *Nitrobacter*) and chemical processes of nitrite oxidation and nitric oxide (NO) production. The model simulations revealed that a narrow and constant pH range is required in the reactor. Stable nitrate production is only achieved at a pH value close to 6. At higher pH values (above 6.5), NOB are too slow to remove the nitrite produced by AOB. At lower pH values, acid-tolerant AOB outcompete acid-sensitive AOB and pH values below 4.5 can be reached. At such low pH values NOB are inhibited by nitrous acid (HNO<sub>2</sub>). While bacterial nitrite oxidation is inhibited, HNO<sub>2</sub> is converted chemically to nitrate and substantial amounts of the volatile intermediate NO are released to the atmosphere. Our computer simulations confirm experimental observations that nitrification of urine requires a narrow pH range, which can be achieved by carefully controlling the urine inflow rate.

[EE-6]

## **Biofilm kinetic modeling and inhibition of ammonia oxidizing bacteria**

**Ellen Lauchnor** (1), Lewis Semprini (2), Brian Wood (2)

*(1) Montana State University; (2) Oregon State University*

Ammonia oxidizing bacteria (AOB) drive the first step of nitrification in the environment and wastewater treatment. When exposed to aromatic hydrocarbons such as phenol and other wastewater contaminants, AOB can become inhibited and stall nitrification. Studies have shown that biofilms provide protection to cells and may achieve more effective wastewater treatment by maintaining AOB activity during contaminant (phenol) exposure. Here, the kinetics of ammonia oxidation and inhibition in biofilms were determined with integrated spatial measurements and reactive transport modeling. In this work, biofilms of the AOB, *Nitrosomonas europaea*, were exposed to phenol while assessing nitrite production in treated and control biofilms. Observed inhibition, measured by percent reduction of activity, was less in biofilms than suspended cultures of *N. europaea*. Microelectrodes were used to measure gradients of dissolved oxygen and pH inside control and phenol exposed biofilms. A reactive transport model of the biofilm was developed and included fluid transport, diffusion within the biofilm, acid-base equilibria and Monod kinetics of ammonia oxidation and phenol inhibition. The model was calibrated to the DO and pH profiles to determine the Monod kinetic parameters of the *N. europaea* biofilms. Under most experimental conditions, the same parameter values were able to fit the rates of ammonia oxidation in both the biofilms and suspended cells. The lower inhibition in biofilms was due to substrate diffusion limitations causing cells to be less active in the biofilm and less susceptible to inhibition. The study demonstrates a method to calibrate kinetic parameters in nitrifying biofilms using microsensor measurements.

[EE-7]

## Challenges encountered calibrating N<sub>2</sub>O dynamics from mixed cultures

**Carlos Domingo-Félez**<sup>1</sup>, Carles Pellicer-Nàcher<sup>1</sup>, Morten S. Petersen<sup>1</sup>, Rosana González-Combarros<sup>1</sup>, Marlene Mark Jensen<sup>1</sup>, Gürkan Sin<sup>2</sup>, Barth F. Smets<sup>1</sup>  
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Nitrous oxide (N<sub>2</sub>O) is a by-product of biological nitrogen removal with a strong environmental impact as a greenhouse gas. Research has focused on conceptualizing our understanding of the complex interrelationships within these systems to lastly develop mitigation strategies. However, the development of accurate process models has been proven difficult as several proposed model structures have failed to describe observations. The present study used a pseudo-mechanistic model distinguishing N<sub>2</sub>O production from autotrophic and heterotrophic bacteria to successfully describe experimental data collected during controlled batch tests with activated sludge biomass. Interestingly, under conditions of no carbon addition experimental and modelling results indicated a strong influence of heterotrophic bacteria on N<sub>2</sub>O production, further confirmed on a different activated sludge biomass. Further, mapping the uncertainty of the model parameters showed a higher uncertainty in the N<sub>2</sub>O predictions compared to other better described nitrogenous species such as ammonium, nitrite or nitrate. Even though the total N<sub>2</sub>O production was not sensitive to certain parameters the contribution of individual pathways was. Unfortunately, an in-depth literature review revealed a high degree of uncertainty in these parameter values and a lack of quality data to accurately assess whether autotrophic or heterotrophic microbial processes contributed more to the total N<sub>2</sub>O pool. Therefore elucidating knowledge gaps encountered during model calibration combined with optimal experimental design will facilitate the development of strategies to minimize the carbon footprint of wastewater treatment plants. This work represents a step further in understanding the N<sub>2</sub>O production and emissions associated to conventional wastewater treatment.

[EE-8]

## **Mimicking annual temperature variations: Response of a partial nitritation/anammox microbial community to different influents.**

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Partial nitritation/anammox (PN/A) processes to treat municipal wastewater has been recently reported. However, these studies mainly focused on the effect of low temperatures during winter, reported as a major challenge. However, attainable performance recovery after a winter period (i.e. temperatures between 10-12°C) is still unknown. The organic carbon (OC) in municipal wastewater will favor denitrification, thus competing with aerobic (AOB) and anaerobic (AnAOB) ammonium oxidizers for oxygen and nitrite, respectively, which has been reported repeatedly. But what about other wastewater constituents? Knowledge about the comprehensive influence of wastewater composition is also required.

A lab scale moving bed biofilm PN/A reactor was operated for one year each with different influents (synthetic wastewater and real wastewater with low content of OC) to follow the behavior of the microbial community. The inferred gradual temperature change mimicked seasonal variations, i.e. 20°C (maximum) to 10°C (minimum).

Change in temperature did influence the abundance of AnAOB for both influents, showing a reduction of 50% compared to initial abundances. Nitrite oxidizer's (NOB) and AOB population were relatively stable in the synthetic wastewater, but in real wastewater the abundance reduced by 60% and 40%, respectively. This behavior corresponds well to the reactor performance. Ammonium conversion rates dropped from 61.5 to 14.2 gNH<sub>4</sub>-Nm<sup>-3</sup>d<sup>-1</sup> (synthetic feed) and 92.6 to 24.7 gNH<sub>4</sub>-Nm<sup>-3</sup>d<sup>-1</sup> (real feed) at 10°C, however, it only recovered for the synthetic feed with some nitrite accumulation during temperature increase.

The results showed that PN/A is possible for moderate climate, however, it is dependent on the wastewater composition.

## Relative abundance of *Nitrotoga* in a biofilter of a freshwater aquaculture plant

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The worldwide increasing seafood consumption leads to high fishing pressure and results in strongly overfished oceans. In this context the use of aquaculture systems gains increasing attention for the production of marine as well as limnic fish and other aquatic animals. In recirculation aquaculture systems (RAS), the nitrification process in moving bed biofilters is essential to maintain fish health. Inside these biofilters ammonia and nitrite oxidizing bacteria (AOB and NOB) colonize carrier elements, forming dense biofilms together with heterotrophic bacteria. The sensitive process of microbial nitrification inside these systems is strongly influenced by changing environmental parameters. In most aquaculture systems (marine and freshwater), *Nitrosomonas* and *Nitrospira* were identified as main ammonia and nitrite oxidizers. In this study a freshwater RAS was used for the production of rainbow trouts and driven at a temperature of 10-14°C and pH 6.8. Community analyses via genus specific PCR, FISH and EM revealed a coexistence of NOB *Nitrospira* and *Nitrotoga*. The latter had a 16S rRNA sequence similarity of 99.9% to cold adapted *Nitrotoga arctica*, which was initially enriched from permafrost-affected soils in Siberia. Thus, we could identify *Nitrotoga* as abundant NOB inside a RAS. Hypothesizing that a slightly acidic pH favors the growth of *Nitrotoga*, we performed experiments with nitrite oxidizing enrichment cultures derived from the nitrifying biofilm. Whereas both NOB were active at a broad range of pH values, *Nitrospira* was out-competed by *Nitrotoga* in long-term incubations at low pH values (5.7 to 6.0). Reference cultures of *Nitrospira defluvii* and the newly isolated *Nitrotoga* were used to confirm separation of both genera into distinct ecological niches with regard to their pH and temperature optima. Besides the low temperature used in this RAS, slightly acidic pH-values seem to favor *Nitrotoga* over *Nitrospira* and can be applied as new isolation criterion for this NOB.

[EE-10]

## Density and distribution of nitrifying guilds in rapid sand filters for groundwater treatment

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Biological rapid sand filtration is a low-cost treatment technology that can efficiently remove  $\text{NH}_4^+$  from groundwater down to drinking water standard levels. In this work we quantified the nitrifying guilds at 5 drinking water treatment plants (DWTPs), and investigated their spatial distribution and role in  $\text{NH}_4^+$  removal from these filters. Molecular quantification by qPCR showed the presence of  $1.1\text{-}16 \times 10^{15}$  total Eubacteria per  $\text{m}^3$  filter material, while ammonium oxidizing bacteria (AOB) comprise about 0.35-12% of the total bacterial community. Nitrospira appeared to be the predominant  $\text{NO}_2^-$  oxidizer, constituting up to 47% of the total bacterial community. Nitrobacter densities constituted at maximum 0.04% of the total bacterial community. The distribution of the targeted microbial guilds was homogeneous across the ca. 0.5 m depth in most filters, except at one treatment plant where density of Eubacteria, AOB, Nitrobacter and Nitrospira decreased steeply with depth. AOB stratification followed the  $\text{NH}_4^+$  gradient in the filter, suggesting correlation of the AOB density with the  $\text{NH}_4^+$  loading. This correlation was consistent also across DWTPs, with higher AOB densities observed at DWTPs operating at higher  $\text{NH}_4^+$  loading rates. Ammonium oxidizing archaea (AOA) were detected at most DWTPs at low densities, but outnumbered AOB in the deeper filter regions when AOB were stratified. These results suggest that bacterial  $\text{NH}_4^+$  oxidation is predominant at high  $\text{NH}_4^+$  loading conditions, whereas archaeal activity is relevant at low loading conditions. Currently, we are seeking confirmation of in situ activity of the detected AOA and are investigating the reasons for the peculiarly high Nitrospira abundance.

[EE-11]

## **Nitrifiers in the Fluidized Sand Biofilter of a Recirculating Aquaculture System**

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Recirculating aquaculture systems (RAS), as nearly closed-loop systems, represent a unique engineered environment to study the nitrogen cycle and minimize environmental N-cycle perturbation by reducing point source aquacultural pollution. The vast majority of nitrogen input into a RAS is from the conversion of feed protein to ammonia and urea by fish in the system. The predominant byproduct of fish protein catabolism is ammonia, which is toxic to the fish and must be removed prior to returning water to the rearing tank. Typically, RAS employ nitrifying biofilters to remove ammonia, and organisms from the genera *Nitrosomonas* and *Nitrobacter* are commonly assumed to be the primary nitrifiers. The UW-Milwaukee School of Freshwater Sciences (SFS) has operated a RAS fluidized sand biofilter without major nitrification disruption since the mid 1990's. In this study, we set out to understand what organisms are facilitating nitrification in the SFS biofilter. The biofilter matrix was studied using high throughput sequencing, end-point PCR, clone libraries, and qPCR. A time course study at the top of the filter matrix indicated ammonia-oxidizing archaea (AOA) and *Nitrospira* spp. were the dominant nitrifying taxa. The biofilter was then sampled at different depths, wherein *Nitrosomonas* spp. were found to co-occur with AOA at greater depths. *Nitrobacter* spp. were absent in both the time series and depth samples. Phylogenetic analysis of *amoA* genes for both archaea and bacteria, in addition to the *nxrB* genes for *Nitrospira* spp., indicate the SFS biofilter harbors novel uncultured nitrifying organisms and raises questions regarding RAS biofilter nitrification optimization.

[EE-13]

## **Modeling simultaneous anaerobic methane and ammonium removal in a granular sludge reactor**

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Anaerobic nitrogen removal technologies offer advantages in terms of energy and cost savings over conventional nitrification-denitrification systems. A mathematical model was constructed to evaluate the influence of process operation on the coexistence of nitrite dependent anaerobic methane oxidizing bacteria (n-damo) and anaerobic ammonium oxidizing bacteria (anammox) in a single granule. The nitrite and methane affinity constants of n-damo bacteria were measured experimentally. The biomass yield of n-damo bacteria was derived from experimental data and a thermodynamic state analysis. Through simulations, it was found that the possible survival of n-damo besides anammox bacteria was sensitive to the nitrite/ammonium influent ratio. If ammonium was supplied in excess, n-damo bacteria were outcompeted. At low biomass concentration, n-damo bacteria lost the competition against anammox bacteria. When the biomass loading closely matched the biomass concentration needed for full nutrient removal, strong substrate competition occurred resulting in oscillating removal rates. The simulation results further reveal that smaller granules enabled higher simultaneous ammonium and methane removal efficiencies.

[EE-14]

## Biokinetics of nitrogen transformation in soil biofilm systems

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Among the various treatments for secondary effluents, using soil infiltration is a common and highly cost effective technology. It is based on the consumption of soluble organic compounds by fixed biomass on the soil's surface (in favor of biomass production or metabolism). This tertiary process purifies the effluents in water to a higher quality that can be reused for unrestricted agriculture and food production. One of the most common habitats for bacterial fixed biomass- biofilms- in nature is on soil. An environmental biofilm community contains many species of bacteria with various functionalities; the biofilm dominant species are those who fit best for the habitat conditions and available nutrients. Thus we can refer to the natural biofilm on soils as a versatile bioreactor that adjusts its functionality by the habitat conditions. The availability of oxygen is a fundamental parameter that defines microbial niche conditions and the dominant N bioreaction which is reflected at the ratios of N compounds.

Depending on the environmental conditions, different bioreaction take place as the effluent percolate through the soil depth. One of the main ones is nitrification and denitrification in dependence on oxygen and substrate availability in addition to the biofilm structure and microniche.

Using soil columns set up, operated in an Upflow- unsaturated mode fed with different N/C ratios synthetic secondary effluent this work is aim to simulate the process and track the biokinetic of the bioreactions by water quality parameters and molecular analysis of the soil's community at different depths. Also, hydrogen peroxide was examined to implement as oxygen source to overcome the oxygen limitations which might change significantly the bacterial community structure, the functional groups abundance and thus the bio-kinetic of the chemical reactions.

The objective of this research is to establish a defined model, based on nitrogen compound bio-kinetics, to examine and compare the effect of effluent oxidation on soil treatments; which may enable higher efficiency of effluent purification while lowering the cost of raising water quality and water reuse in soil treatments.

[EE-15]

## **A high-rate nitrification bioreactor at 50°C opens up opportunities for thermophilic wastewater treatment**

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Several thermophilic nitrogen converting micro-organisms have been enriched from natural environments such as hot springs or hydrothermal vents. However, until now, no continuous cultivation systems are described for the treatment of nitrogen in hot wastewaters although this could offer different advantages compared with the existing mesophilic nitrogen removal processes. In this study, samples from composting facilities served as inocula for the batch-wise enrichment of thermophilic (50°C) nitrifying communities. Subsequently, the enrichments were transferred to a sequential batch reactor fed with synthetic influent containing ammonium and nitrite, reaching nitrification rates of  $161 \pm 19$  and  $244 \pm 20$  mg N L<sup>-1</sup> day<sup>-1</sup> at 50°C, for ammonium and nitrite respectively. The biomass showed high specific nitrifying activities of up to  $198 \pm 10$  and  $894 \pm 81$  mg N g<sup>-1</sup> VSS day<sup>-1</sup> for ammonium and nitrite oxidation, respectively. 16S rRNA gene Illumina sequencing showed up to 17% ammonium oxidizing archaea (AOA) related to *Nitrososphaera gargensis* (99%), no AOB and 25% nitrite oxidizing bacteria (NOB) related to *Nitrospira calida* (98%). This unique AOA/NOB continuous reactor culture was physiologically characterized, resulting in a clear optimal activity at 50°C for the NOB, while the AOA had a broader temperature optimum (45-55°C). The NOB were much more sensitive for both free ammonia and free nitrous acid than the AOA, opening opportunities for NOB inhibition strategies to reach more cost beneficial nitrogen removal processes such as nitrification/denitrification or deammonification. Overall, this study for the first time describes the enrichment of autotrophic thermophilic nitrifiers from aerobic compost and the successful operation of a thermophilic nitrifying bioreactor, opening up opportunities for thermophilic nitrogen removal in warm types of wastewater.

[EE-16]

## **A flow cytometry–fluorescence in situ hybridization method to detect ammonia oxidizing bacteria (AOB) under low dissolved oxygen**

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Nitrifying engineered systems are operated with a high level of aeration due to the aerobic metabolism of ammonia oxidizing bacteria (AOB) with implications in the operational cost and carbon footprint. However, we previously reported complete nitrification in a chemostat with dissolved oxygen lower than 0.5 mg/l. Such conditions seemed to promote the selection of AOB communities with high yield growth rate (1.5 mgVSS AOB/mg NH<sub>4</sub><sup>+</sup>-N), which might be a result of mixotrophic metabolism. The aim of this study is to understand how the AOB change their metabolism under low oxygen levels by metagenomic studies. For that reason, we developed a method combining in-solution fixation-free fluorescence in situ hybridization (FISH) with fluorescence-activated cell sorting (FACS). Several combinations of different dyes were tested to detect simultaneously AOB (Cy3, Cy5) and total bacteria (FITC, SYTO 9). The method was calibrated using samples of activated sludge collected from a nitrifying municipal wastewater. Challenges in FACS detection were encountered, probably related to the low relative abundance of AOB present in the samples and/or low fluorescent signals. However, after confirming the presence of two populations, those cells were sorted using one-stage sort. The feasibility of FISH–FACS to separate targeted uncultivated populations was therefore demonstrated, though optimization of the protocol is still needed. The developed method is now applied to analyze and compare the AOB communities grown in four parallel nitrifying continuous flow reactors which are operated at low and high aeration, 2% and 21% respectively.

[EE-17]

## **Effect of aeration condition on nitrous oxide emissions from conventional activated sludge process**

**Atsuko Michinaka**, Ikuo Tsushima, Manabu Matsuhashi, Ryota Kawasumi, Hiroyuki Shigemura, Hiromasa Yamashita

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Nitrous oxide (N<sub>2</sub>O), a greenhouse gas (GHG) is considered to have an impact about 300 times greater than CO<sub>2</sub> on climate change, and emitted from biological wastewater treatment processes. According to a field survey in Japan, conventional activated sludge (CAS) processes were observed higher N<sub>2</sub>O emissions than biological nitrogen removal systems (A<sub>2</sub>O, modified Bardenpho process, etc.). Thus, we investigated an N<sub>2</sub>O gas reduction method in CAS. In this study, to estimate the effect of aeration conditions on N<sub>2</sub>O emissions in CAS, we operated bench-scale continuous flow reactors with raw municipal sewage, and compared N<sub>2</sub>O emissions under full and restricted aeration conditions. The reactors were operated for 35 days; the initial 18 days were under full aeration, and the following 17 days under restricted aeration (a modified CAS involving low air supply in the upstream side of the aeration tank). The quality of influent water was stable during the operation period. The TOC concentration of the effluent in both conditions was less than 10 mg/L, and the removal ratio was more than 70%. The amount of N<sub>2</sub>O emitted under full and restricted aeration conditions were 541.0 and 29.5 mg·N/m<sup>3</sup>, respectively. After changing the aeration conditions, the amount of N<sub>2</sub>O emission reduced significantly while the nitrogen removal ratio increased 16.5 %. The result indicated that control of aeration in CAS could control GHG emissions by reducing not only CO<sub>2</sub> emissions attributable to energy consumption by aeration, but also N<sub>2</sub>O emissions.

## **TOPIC 6: ANAEROBIC PROCESSES OF THE NITROGEN CYCLE**

POSTER & TALK: Tuesday Session, 5-6PM, CCIS L1-140

[AP-1]

### **Diversity, Distribution and Abundance of Nitrite-dependent Anaerobic Methane Oxidation Bacteria in the Coastal and Ocean Sediments**

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Nitrite-dependent anaerobic methane oxidation (n-damo) process is unique in linking the microbial carbon and nitrogen cycles, but the presence of n-damo bacteria in marine ecosystems and the associated environmental factors are still poorly understood. In the present study, detection of n-damo bacteria using 16S rRNA and pmoA gene-based PCR primers was successfully employed to reveal their diversity and distribution in the surface and subsurface sediments of the South China Sea (SCS) and the coastal Mai Po wetland (MP). The abundances of n-damo bacteria were also quantified using 16S rRNA primers. From the continental shelf to the deep abyss, the widespread occurrence of n-damo bacteria with high diversity has been confirmed in both the surface and subsurface sediments of the west Pacific Ocean in this study. The pmoA gene-amplified sequences clustered within three newly erected subclusters, suggesting the unique niche specificity of n-damo bacteria in the marine habitats. Unlike the other two so far known n-damo communities from coastal areas, the pmoA gene-amplified sequences from MP clustered not only in some freshwater subclusters, but also within the three marine subclusters mainly, indicating high complexity of n-damo bacteria in Mai Po wetland. Results suggested that vegetation influences the on n-damo bacterial distribution and community structures and the coexistence of denitrifying and sulfate-reducing methanotrophs in MP. The relationship between n-damo and communities of ammonia-oxidizing prokaryotes (AOA, AOB and anammox) were investigated in bioturbated faunal burrows. Finally, the environmental factors associated with n-damo bacterial communities were also analyzed and revealed at these two environments.

[AP-2]

## **A gas-permeable membrane biofilm reactor enriches highly active N<sub>2</sub>O-reducing bacteria for isolation**

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Nitrous oxide (N<sub>2</sub>O) is one of powerful greenhouse gases and most serious ozone-depleting compounds in the 21st century. Harnessing N<sub>2</sub>O reducing bacteria could be promising in that they are able to mitigate N<sub>2</sub>O emission from cultivated soils and wastewater treatment plants (WWTPs). However their phylogenetic and physiological traits are still elusive. Therefore, the objectives of this study are to enrich and isolate highly active N<sub>2</sub>O reducing bacteria. For effective enrichment of N<sub>2</sub>O reducing bacteria, a gas-permeable membrane biofilm reactor (GPMBfR) was developed. A GPMBfR allows counter-current supplies of organic carbon and N<sub>2</sub>O without bubble formation via a gas-permeable membrane where a biofilm is grown. A GPMBfR was fed with activated sludge from a municipal WWTP as an inoculum and operated for 191 days. Deep sequencing of 16S rRNA gene illuminated that the predominant species in the GPMBfR are affiliated with the family Rhodocyclaceae. Out of 115, 152 isolated strains, classified to the genera *Azospira*, *Dechloromonas*, and *Azoarcus* within the family Rhodocyclaceae, were acquired from the enriched biomass in the GPMBfR. Contrary, these isolate strains were not obtained by isolation from the inoculum, underscoring significance of the enrichment procedure. The strains showed high affinity for N<sub>2</sub>O, enough to reduce the level of N<sub>2</sub>O concentration in a WWTP. Furthermore, the strain affiliated with the genus *Dechloromonas* sp. had the highest N<sub>2</sub>O reducing activity ( $1.63 \times 10^{-2}$  pmol/cell/h) among the isolates within the family Rhodocyclaceae. Continuous cultivation by a GPMBfR successfully enriched active N<sub>2</sub>O reducing bacteria and significantly improved their probability of their isolation.

[AP-3]

## Counter-diffusion biofilm for simultaneous nitrification and denitrification reduces N<sub>2</sub>O emission: Depth-profile analysis

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A membrane-aerated biofilm reactor (MABR) allows effective simultaneous nitrification and denitrification because of its inherent counter-diffusion biofilm geometry where oxygen is supplied from a gas-permeable membrane, i.e. the bottom of a biofilm. In case of wastewater containing organic carbon and ammonia, the counter-diffusion geometry creates an environment where organic carbon concentration is the maximum with minimal dissolved oxygen (DO) concentration, improving denitrification performance. Given the trait, we hypothesized that a counter-diffusion biofilm reduces N<sub>2</sub>O emission than a conventional biofilm. To verify this hypothesis, two laboratory-scale reactors with the same dimension except biofilm geometries, MABR and conventional biofilm reactor (CONV), were operated to investigate depth profiles of dissolved gases (DO, NO and N<sub>2</sub>O) and bacterial community by microsensors, real time qPCR and 16S rRNA gene amplicon sequencing. Total nitrogen removal efficiency was slightly higher in MABR ( $33.6 \pm 3.3\%$ ) than in CONV ( $28.1 \pm 4.9\%$ ). The depth profiles of N<sub>2</sub>O and NO indicated that those emissions from the MABR biofilm were much lower than those from CONV biofilm ( $0.16 \pm 0.12$  mgN<sub>2</sub>O-N/L in MABR vs.  $2.8 \pm 0.16$  mgN<sub>2</sub>O-N/L in CONV biofilm). Higher abundances of nirS, nirK and nosZ genes were observed throughout the biofilm in MABR, which tallies with the lower NO and N<sub>2</sub>O levels. More diversified microbial communities were formed in MABR than in CONV. In sum, the MABR embraced an environment with higher abundances of denitrifying bacteria and likely reduced N<sub>2</sub>O, which could be an innovative and promising technology for nitrogenous wastewater treatment achieving lower N<sub>2</sub>O emission.

[AP-4]

## Denitrification in mountain lakes from the Pyrennes

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Increased nitrogen (N) deposition is affecting biogeochemical cycling and biota in high-altitude oligotrophic lakes in mountain areas on the northern hemisphere. In the Pyrenees, the amount of reactive N has been increasing since the 1950's, but the potential for N transformations and return of N to the atmosphere via denitrification in these mountain lakes are poorly understood. Ten lakes covering the environmental variability among the Pyrenean lakes were investigated to determine actual and potential denitrification activity in intact core sediments using the acetylene inhibition technique combined with microsensors for nitrous oxide. The genetic potentials for denitrification and ammonia oxidation in the sediments and epilithic biofilms were assessed by quantifying the gene pools of the two structurally dissimilar nitrite reductases (*nirS* and *nirK*) and the bacterial and archaeal *amoA* genes, respectively. The actual rates varied greatly both within a single lake and among lakes, with a mean actual rate of  $2.4 \pm 2.3 \mu\text{mols N}_2\text{O m}^{-2} \text{ h}^{-1}$ . Denitrification was nitrate limited. Nitrite reductases gene copies were more abundant than *amoA* genes. These findings suggest that lake capacity to remove nitrate has not been saturated under conditions of increased N deposition.

[AP-5]

## **Implications of anaerobic nitrogen-transformations in tailings' biogeochemical processes**

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Oil sands tailings ponds are known to generate large volumes of biogenic methane, a potent greenhouse gas, produced during the biodegradation of residual hydrocarbons entrained in tailings. Methanogenic activity is thought to be hampered when bioavailable nitrogen is limited for microbial growth. Sustenance of methanogenic activities in oil sands tailings under nitrogen depleted conditions prompted to investigate the mechanisms of nitrogen availability for microbial functions. To simulate the nitrogen depleted or available conditions, the cultures consisting of mature fine tailings (MFT) collected from tailings ponds combined with methanogenic media containing or devoid of available nitrogen under anaerobic N<sub>2</sub> headspace were established in microcosms and amended with the bitumen extraction aid citrate, as a model carbon source. After a longer lag phase in nitrogen deficient cultures, equivalent methane production was observed in the microcosms regardless of the presence or absence of nitrogen in the medium. Acetylene reduction and <sup>15</sup>N<sub>2</sub> incorporation into microbial biomass suggested the occurrence of N<sub>2</sub>-fixation concurrent with methanogenesis. Further analyses have revealed the presence of functional genes involved in N<sub>2</sub>-fixation and denitrification suggesting the role of anaerobic nitrogen transformations in biogeochemical processes in tailings ponds. Understanding these processes is important for effective management of oil sands tailings. The results might have implications in assessing the feasibility of end-pit lakes designed to naturally attenuate large volumes of MFT under cap water. However, sustained methanogenic activity in end-pit lakes could impact contaminant partitioning to the cap water. These possibilities will be further investigated during our ongoing laboratory experiment.

[AP-6]

## **IC50 values for 14 substances on anammox activity using a <sup>15</sup>N tracer technique**

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Potential inhibitory effect of 21 substances on the anammox activity was examined with a simple batch test using a <sup>15</sup>N tracer technique. Five substances did not show any adverse effect, but 16 substance were inhibitory. For 14 substances, IC50 were determined; 0.22-0.28 mM for 2 heavy metals, 0.07-2.6 mM for 4 chelators, 9 -15 mM for 4 amines and 13.5 - 90 mM for 4 organic solvents. EDTA, most commonly used chelator for dissolving metals in water, has the strongest adverse effect. This suggests that, if EDTA has been employed as a chelator for dissolving heavy metal species for examining their potential inhibitory effects in a previous study, the result obtained may not solely be attributed to heavy metals, but may be due to EDTA. To avoid ambiguous result which could be created by mixing EDTA and a heavy metal species, each heavy metal species was dissolved in the assay solution without employing EDTA for aforementioned experiments. In parallel, we examined potential inhibitory effect of Zn<sup>2+</sup> using 2 assay solutions with and without EDTA to see if EDTA may affect. It was demonstrated that Zn<sup>2+</sup> showed adverse effect at a similar level, irrespective of adding EDTA to the assay solution. Similar results were obtained for Cu<sup>2+</sup>. These results suggest that, apparently, EDTA may not adversely affect in examining effect of heavy metals. However, from another point of view, these metals examined may alleviate an adverse effect of EDTA.

[AP-7]

## Is the isotopic site preference in N<sub>2</sub>O conservative? Evidence for isotopic fractionation during N<sub>2</sub>O production during denitrification.

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Resolving the microbial origin of N<sub>2</sub>O has been a long-standing focus of research for many decades. In the last 15 years, site preference (SP: the difference in  $\delta^{15}\text{N}$  of the central and outer N atoms in N<sub>2</sub>O) has emerged as a conservative tracer for distinguishing N<sub>2</sub>O from denitrification and nitrification (hydroxylamine oxidation). Applications of SP as a conservative tracer are based on microbial culture data that shows differences in SP between N<sub>2</sub>O produced from denitrification (-10 to 0 ‰) and nitrification (30-35 ‰) and the lack of fractionation in SP during N<sub>2</sub>O production in pure culture. We present data on the isotopic composition of N<sub>2</sub>O produced by two species of denitrifiers, *Pseudomonas chlororaphis* and *Pseudomonas aureofaciens* that lack N<sub>2</sub>O reductase. All cultures were provided with 10  $\mu\text{mole}$  nitrate or nitric oxide as the substrate and two different levels of succinate (1 and 25 mM) as an electron donor to control the velocity of denitrification. We confirm an inverse relationship between isotopic fractionation during N<sub>2</sub>O production and velocity, and we document, for the first time, fractionation in SP in *P. chlororaphis* of 2.3 and 11 ‰ at succinate concentrations of 25 and 1 mM, respectively. The implications of these results on N<sub>2</sub>O source tracing will be discussed.

[AP-8]

## Spatiotemporal Discrimination of Bacterial Communities Harboring *nosZ* Genes over a Three-Year Period in Two Contrasting Agricultural Soils

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Diverse atypical (atyp) *nosZ* genes encoding N<sub>2</sub>O reductase are found in soils, established recently from metagenome surveys and clone libraries. Atyp *nosZ* dominate in abundance over typical (typ) *nosZ* genes found in many well-studied denitrifiers, suggesting the functional relevance of the former that has yet to be fully demonstrated. Here, we report the dynamics of *nosZ* communities in a three-year study at two contrasting agricultural fields in Illinois (sand vs. silt loam), both under conventionally managed corn/soybean rotations in similar climate. Nine soil cores (0-30 cm) per site were sampled 4-5 times each year at spatial scales representing location (km), within-field centroids (m, cm), and depths (0-5-, 5-20-, 20-30 cm). Community T-RFLP of PCR-amplified atyp- and typ *nosZ* and 16S rRNA genes, along with amplicon-based sequences (MiSeq) of the 16S rDNA were used in conjunction with soil physicochemical and climate data to discriminate community changes according to spatial- and temporal factors. Among factors for all genes, the two fields were always distinct (n=652, R>0.4, p=0.1%) regardless of time and depth, and soil conditions (e.g. Temp., N, Mg) drove stronger distinctions in communities than seasonal crop or management timing (i.e. active crop, post-harvest) alone. Within field plots, communities shifted temporally and while crop-dependent, maintained depth discrimination, especially between topmost and deepest layers (R=0.33-0.83, p=0.1%). Of 13 variables tested, the magnitude of soil temperature flux rather than temperature averages was correlated most frequently to *nosZ* community dynamics. While overall beta-diversity separate the two sites, homologous *nosZ* genes are shared. Seasonal shifts in specific TRFs of atyp *nosZ* genes suggest differential responses occurring within the functional community, and putatively match sequences found dominant in the metagenome pools. Consistent among dominant taxa found, the most abundant atyp *nosZ* in both soils include *Anaeromyxobacter* (delta-Proteobacteria) and *Opitutus* (Verrucomicrobia). Overall, spatiotemporal shifts in *nosZ* communities follow temporal changes in soil conditions and parallel overall bacterial community dynamics, but specific *nosZ* populations exhibit responses to temporal soil conditions partitioned by depth.