ICoN8

International Conference on Nitrification and Related Processes

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ICoN8 Abstract Booklet

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Session: Opening Plenary
Presenter: Holger Daims
Title: An expanded picture of nitrification: comammox, alternative lifestyles, and microbial interactions
Authors: Daims, Holger, University of Vienna, Centre for Microbiology and Environmental System Science (CeMESS), Division of Microbial Ecology (DoME), Vienna, Austria

According to established textbook knowledge, the nitrifying microorganisms are specialized, aerobic chemolithoautotrophs that oxidize either ammonia or nitrite and are quite restricted in their metabolic versatility beyond nitrification. This perspective on the nitrifiers has changed dramatically in recent years following the discoveries of alternative energy metabolisms, complete ammonia oxidizers (comammox), and extended interactions of nitrifiers with other microbes. In particular, the utilization of organic nitrogen compounds appears to be an ecophysiologically important and niche-defining feature of certain nitrifiers. Such relevant compounds include urea, cyanate, and – as recently discovered – guanidine, a chemically stable nitrogen compound that occurs widely in nature and has numerous industrial applications. Guanidine is used by microbes as a nitrogen source, but microorganisms growing on guanidine were not known. Recently, we found that the comammox species *Nitrospira inopinata* and likely most other comammox organisms grow on guanidine as the sole source of energy, reductant, and nitrogen. Proteomics, enzyme kinetics, and the crystal structure of a previously unidentified *N. inopinata* guanidinase homologue demonstrated that it is indeed a *bona fide* guanidinase. Transcription of comammox guanidinases was induced in wastewater treatment plant microbiomes upon incubation of activated sludge with guanidine, suggesting that nitrification by comammox is a key pathway for guanidine degradation. The discovery of guanidine as selective substrate shows a unique niche of comammox organisms and may offer new opportunities for their isolation, biodegradation of micropollutants, and manipulation of nitrifier communities in agriculture to reduce greenhouse gas emissions.

Session: Biochemistry/Bioenergetics
Presenter: Thomas Barends
Title: Inside the Anammoxosome: Molecular Details of Anaerobic Ammonium Oxidation
Authors: Barends, Thomas, R.M., Max Planck Institute for Medical Research

Our understanding of the global nitrogen cycle was changed dramatically by the discovery of anaerobic ammonium oxidizing (anammox) bacteria in the 1990s. Indeed, anammox bacteria are of global importance and are now believed to be responsible for up to 30 to 70% of the total yearly nitrogen removal from the oceans. These organisms combine ammonium and nitrite to form dinitrogen gas in a specialized intracellular compartment called the anammoxosome. Strikingly, the central anammox metabolism relies on use the extremely reactive compound hydrazine as a central intermediate. To elucidate how bacteria carry out such extraordinary biochemistry, we have studied the structures of key redox enzyme complexes in this process. Among these is a nitrite/nitrate oxidoreductase (NXR) homologous to the NXRs found in nitrifying bacteria. Despite NXR's central role in the biogeochemical nitrogen cycle, no structural information on NXRs has been available for decades. Here we present a multiscale molecular model of the anammox NXR, obtained by combining crystallography, cryo-electron tomography, and helical reconstruction single-particle cryo-EM. Together with in vitro reconstitution studies and enzyme kinetics, our results show that, in contrast to the membrane-bound NXRs from nitrifiers, anammox-specific NXR forms tubule-like structures spanning the anammoxosome that are held together by a newly discovered subunit we call NXR-T. The structure has implications for the reaction mechanism as well as the interactions with redox partners.

Session: Biochemistry/Bioenergetics
Presenter: Kyle Lancaster
Title: Maturation and Reactivity of Cytochrome P460
Authors: Lancaster, Kyle M., Cornell University, Ithaca, NY, USA

Cytochrome P460 is a small enzyme bearing eponymous heme P460 cofactors that carry out the selective oxidation of hydroxylamine. This lecture will discuss the autocatalytic maturation of the cyt P460 heme P460 cofactor, showcase the numerous molecular architectural features that imbue reactivity, and propose a means by which these cofactors can complete the 4-electron oxidation of hydroxylamine in ammonia oxidizing bacteria.

Session: Biochemistry/Bioenergetics
Presenter: Gordon Williamson
Title: Site-seeing in ammonia monooxygenases
Authors: Williamson, Gordon, and Lehtovirta-Morley, Laura, School of Biological Science, University of East Anglia, Norwich Research Park, Norwich, NR47TJ, UK

Introduction: Ammonia oxidising microorganisms (AOMs) facilitate ammonia oxidation, the first step of nitrification and an integral component of the global biogeochemical cycle. However, their activity also siphons ammonia-based fertiliser from agricultural environments and directs it into the nitrogen cycle. This generates climate-active gases and threatens food security. To reduce the loss of fertiliser and balance the nitrogen cycle, it is essential to enhance our understanding of AOM-mediated ammonia oxidation. Bacterial and archaeal AOM both use ammonia monooxygenases (AMO) to oxidise ammonia, but historical issues with purification have limited study of AMO structure and function. In particular, the location and architecture of the active site remains elusive. The advent of AlphaFold2 represents an avenue for investigating the active site in the absence of crystal structures.

Key results:

1. Structural differences between bacterial and archaeal AMO revealed by AlphaFold2.
2. Putative active sites identified via molecular docking simulations.
3. Alkyne inhibitors could be used to experimentally validate the active site.

Concluding statement: Here we demonstrate an in silico approach to gain insight into the active site architecture of the challenging and biologically relevant AMO proteins, and present a proteomics-based methodology for testing those insights in vitro.

Keywords: ammonia monooxygenase, ammonia oxidation, nitrification, nitrogen cycling, alphafold

Session: Biochemistry/Bioenergetics
Presenter: Wei Qin
Title: Differential substrate affinity and catabolite repression enable preferential use of urea by ammonia-oxidizing bacteria

Authors: Qin, Wei, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Wei, Stephany, P, Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA; Zheng, Yue, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian, China; Choi, Eunkyung, Department of Microbiology and Cell Science, University of Florida, Davie, Florida, USA; Li, Xiangpeng, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Johnston, Juliet, Lawrence Livermore National Laboratory, Livermore, California, USA; Wan, Xianhui, Department of Geosciences, Princeton University, Princeton, New Jersey, USA; Abrahamson, Britt, Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA; Flinkstrom, Zachary, Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA; Wang, Baozhan, College of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu, China; Li, Hanyan, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Hou, Lei, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Tao, Qing, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Chlouber, Michael, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Sun, Xin, Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA; Wells, Michael, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Ngo, Long, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Hunt, Kristopher, A., Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA; Urakawa, Hidetoshi, Department of Ecology and Environmental Studies, Florida Gulf Coast University, Fort Myers, Florida, USA; Tao, Xuanyu, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Wang, Dongyu, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Wang, Dazhi, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian, China; Pan, Chongle, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Weber, Peter, K, Lawrence Livermore National Laboratory, Livermore, California, USA; Jiang, Jiandong, College of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu, China; Zhou, Jizhong, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA; Zhang, Yao, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian, China; Stahl, David, A, Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA; Ward, Bess, B, Department of Geosciences, Princeton University, Princeton, New Jersey, USA;

Mayali, Xavier, Lawrence Livermore National Laboratory, Livermore, California, USA; Martens-Habbena, Willm, Department of Microbiology and Cell Science, University of Florida, Davie, Florida, USA; Winkler, Mari -Karoliina, H, Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA.

Four distinct lineages of ammonia-oxidizing microorganisms (AOM) collectively drive one of the largest nitrogen fluxes in the global nitrogen budget. AOM possess widely different affinities for ammonia, which are thought to largely determine their niche differentiation. Nevertheless, ammonia-oxidizing archaea and bacteria (AOA, AOB), and complete ammonia oxidizers (comammox) commonly co-occur in various ecosystems, suggesting that other factors must be important in coexistence of these lineages. Here, we show that representatives of four AOM lineages employ distinct regulatory strategies for ammonia or urea utilization, thereby minimizing direct competition for either substrate. AOA and comammox preferentially used ammonia over urea, while beta-proteobacterial AOB preferentially used urea, repressed ammonia transport in the presence of urea, and showed higher affinity for urea than ammonia; whereas gamma-proteobacterial AOB co-utilized two substrates. Stable isotope tracing, kinetics, and transcriptomics experiments revealed that both assimilation and oxidation of ammonia are transport-dependent. Our findings reveal novel mechanisms of nitrogen metabolism regulation and transporter-based affinity underlying the contrasting niche adaptation and coexistence patterns among the major AOM lineages.

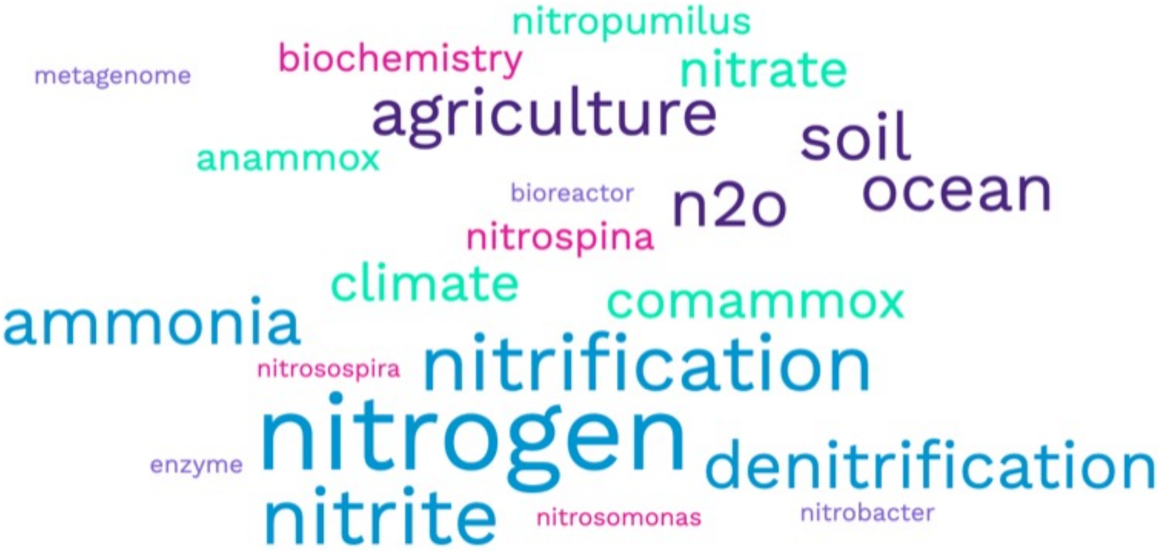
Session: Biochemistry/Bioenergetics
Presenter: Paloma Garrido-Amador
Title: Enrichment and characterization of nitric oxide-reducing microorganisms in a continuous bioreactor

Authors: Garrido-Amador, Paloma, Microbial Physiology Group, Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany; Stortenbeker, Niek, Microbial Physiology Group, Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany; Wessels, Hans, J.C.T., Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud University Medical Center, Geert Grooteplein-zuid 10, 6525GA Nijmegen, The Netherlands; Speth, Daan, R., Microbial Physiology Group, Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany; Garcia-Heredia, Inmaculada, Microbial Physiology Group, Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany; Kartal, Boran, Microbial Physiology Group, Max Planck Institute for Marine Microbiology, Celsiusstraße 1, 28359 Bremen, Germany; School of Science, Constructor University, Campus Ring 1, 28759, Bremen, Germany

Nitric oxide (NO) is a highly reactive and climate-active molecule that, although toxic for many microorganisms, constitutes a key intermediate in the microbial nitrogen cycle. Despite its role in the evolution of denitrification and aerobic respiration, high redox potential, and capacity to sustain microbial growth, our understanding of NO-reducing microorganisms remains limited due to the absence of NO-reducing microbial cultures obtained from the environment directly using NO as substrate. Here, using a continuous bioreactor inoculated with sludge from a municipal wastewater treatment plant and constant supplies of NO as electron acceptor and formate as electron donor, we enriched and characterized a microbial community dominated by two new microorganisms that can grow at nanomolar NO concentrations and also survive micromolar amounts of NO, reducing it to N2 with barely any production of the greenhouse gas N2O. Metagenomic, metatranscriptomic and metaproteomic analyses revealed that in the NO-, formate-fed bioreactor, they used cNOR-like and clade II nosZ proteins and expressed nitrite and nitrate reductases. Interestingly, formate dehydrogenases (FDH) were not found in the complete genome of the most abundant (>80%) microorganism. Together with the high formate oxidation rates in the bioreactor, this suggests that this microorganism oxidizes formate using a hitherto unknown FDH, potentially another molybdopterin-containing protein such as one of the highly expressed nitrate reductases. This study provides new insight into the cultivation and physiology of NO-reducing microorganisms, presenting new opportunities to investigate microbial turnover of this climate-active molecule in non-model microorganisms and its potential application in NOx removal from wastewater.

Session: Biochemistry/Bioenergetics
Presenter: Beate Kraft
Title: Physiology of ammonia-oxidizing archaea under oxygen depletion
Authors: Kraft, Beate, Nordcee, Department of Biology, University of Southern Denmark, Odense, Denmark; Hernández-Magaña, Elisa, Nordcee, Department of Biology, University of Southern Denmark, Odense, Denmark; García-Otero ,Paula, Nordcee, Department of Biology, University of Southern Denmark, Odense, Denmark

The energy metabolism of ammonia-oxidizing archaea (AOA), the oxidation of ammonia to nitrite, requires oxygen. Nevertheless, ammonia-oxidizing archaea are abundant in environments, in which oxygen is undetectable. Their role in these environments has been an enigma until a novel metabolic pathway was discovered in the ammonia-oxidizing archaeon Nitrosopumilus maritimus: After oxygen depletion, N. maritimus produces oxygen and dinitrogen with nitric oxide and nitrous oxide as intermediates. Part of the produced oxygen is directly re-used for ammonia oxidation. This keeps oxygen levels in the nanomolar range, thus evading detection with oxygen sensing methods standardly used to study AOA physiology. By performing incubation experiments in gastight glass vessel using trace oxygen sensors, we show that the proposed NO-dismutation pathway is, apart from N. maritimus, present in several other AOA isolates from the marine environment and from soil. Based on 15N-labelling experiments new insights into the roles of nitric oxide and nitrous oxide as intermediates of the pathway are provided. While all tested isolates are capable of oxygen production, only some AOA produce dinitrogen. Others seem to lack the nitrous oxide reduction step. Furthermore, oxygen accumulation dynamics differ between isolates. In conclusion, dark oxygen production is widely distributed among AOA isolates albeit with important physiological differences between the AOA strains.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Session: Biochemistry/Bioenergetics
Presenter: Britt Abrahamson
Title: Hydroxylamine driven nitrate reduction: A novel anoxic pathway in the complete ammonia oxidizing bacteria Nitrospira inopinata

Authors: Abrahamson, Britt, University of Washington, Seattle, WA, USA; Wan, Xianhui, Princeton University, Princeton, NJ, USA; Candry, Pieter, University of Washington, Seattle, WA, USA; Hunt, Kristopher, A., University of Washington, Seattle, WA, USA; Wang, Dongyu, University of Oklahoma, OK, USA; Flinkstrom, Zach, University of Washington, Seattle, WA, USA; Pan, Chongle, University of Oklahoma, OK, USA; Qin, Wei, University of Oklahoma, OK, USA; Ward, Bess, B., Princeton University, Princeton, NJ, USA; Winkler, Mari, K.H., University of Washington, Seattle, WA, USA

Metabolic pathways allowing aerobic nitrifiers to survive anoxic transitions have been demonstrated for most nitrifying lineages, except complete ammonia oxidizing bacteria (comammox). While comammox species have the genomic potential for a novel pathway coupling the oxidation of hydroxylamine (an ammonia oxidation intermediate) with nitrate reduction, experimental evidence for this reaction in a nitrifying organism has been lacking. Here, we demonstrate hydroxylamine driven nitrate reduction catalyzed by Nitrospira inopinata, the only current comammox isolate, and we explore the physiology of this novel pathway. When anoxic incubations of N. inopinata were conducted in the presence of hydroxylamine and nitrate, nitrite accumulation coupled to hydroxylamine driven nitrate reduction was observed. Abiotic filtrate and heat-killed controls did not accumulate nitrite, but abiotic hydroxylamine decomposition was observed. The absence of nitrite accumulation in the controls confirmed the biotic nature of the hydroxylamine driven nitrate reduction pathway. Relative flux through the biotic nitrate reduction pathway was found to correlate negatively with hydroxylamine concentrations, while abiotic hydroxylamine decomposition was promoted by elevated hydroxylamine concentrations. Nitrate based stable isotope tracking confirmed the fate of nitrate and validated hypothesized reaction stoichiometries. Kinetic analysis demonstrated the pathway could be viable under environmentally relevant concentrations and outcompete abiotic hydroxylamine decomposition. Finally, proteomic analysis identified key functional shifts between hydroxylamine-driven nitrate reduction and aerobic growth. Overall, use of the novel hydroxylamine driven nitrate reduction allows N. inopinata to anoxically gain energy under environmentally relevant conditions.

Session: Biochemistry/Bioenergetics
Presenter: Wouter Lenferink
Title: Alcaligenes faecalis accumulates hydroxylamine as a central intermediate during heterotrophic nitrification

Authors: Lenferink, Wouter, B, Department of Microbiology, Radboud University, the Netherlands; Lazic, Marina, Biological Sciences, University of Alberta, Canada; Jetten, Mike, SM, Department of Microbiology, Radboud University, the Netherlands; Stein, Lisa, Biological Sciences, University of Alberta, Canada; Van Kessel, Maartje, AHJ, Department of Microbiology, Radboud University, the Netherlands; Lücker, Sebastian, Department of Microbiology, Radboud University, the Netherlands

Despite numerous studies conducted on heterotrophic nitrifiers, our understanding of the underlying mechanism remains limited. Whilst strictly depending on organic carbon respiration, heterotrophic nitrifiers can convert ammonium via hydroxylamine to nitrite or nitrate and have been reported to form nitric oxide, nitrous oxide, and dinitrogen gas. Here, we investigated the heterotrophic nitrifier Alcaligenes faecalis to elucidate the mechanism, genetic basis, and physiological benefit of nitrification during carbon respiration. A. faecalis was incubated with isotopically labelled substrates and at different carbon-to-nitrogen ratios. For all culture conditions, we determined growth yield, soluble inorganic nitrogen, and gas production kinetics, as well as gene expression and metabolite production. We showed that A. faecalis produces hydroxylamine, nitrite, nitrous oxide, and dinitrogen gas under a wide range of carbon-to-nitrogen ratios. In contrast to previous reports, the growth yield of A. faecalis did not increase during nitrification. Furthermore, the production of nitrogen gases was likely due to chemical reactions between hydroxylamine, nitrite, and nitric oxide. Recently, genes responsible for nitrogen gas formation from hydroxylamine were described for Alcaligenes ammonioxydans. However, despite the upregulation of this gene cluster, hydroxylamine accumulated in reactor cultures. These findings challenge the view of combined nitrification and denitrification and energy conservation from heterotrophic nitrification in A. faecalis. Furthermore, the excretion of hydroxylamine in a state of nitrogen excess raises questions about the benefit of this physiology. Considering the antibiotic properties of hydroxylamine, we hypothesize that this production is a competitive strategy, and that all other products are due to abiotic or detoxifying reactions.

Session: Biotechnology/Applications
Presenter: Mari Winkler
Title: Unlocking the power of comammox and AOA to enhance engineered applications
Authors: Winkler, M., Civil and Environmental Engineering, University of Washington, Seattle, WA, USA

Anaerobic ammonia oxidation (Anammox) has revolutionized the global nitrogen cycle and its impacts on natural and man-made systems are far reaching. Anammox relies on a constant nitrite and ammonia supply to metabolize both to dinitrogen gas. This talk will demonstration experimental evidence and modelling approaches to elucidate the diffident microbial interactions that can sustain a steady nitrite supply to Anammox via different microbial partners (such as ammonium oxidizing bacteria and archaea, complete ammonium oxidizing bacteria, partial denitrifying bacteria, as well as nitrate driven methane oxidizers) and will highlight how Anammox can interface with novel metabolic pathways including methanol, urea, hydroxylamine, and formate utilization.

Session: Biotechnology/Applications
Presenter: Mengsheng Jia
Title: Exploring the limits of novel thermophilic anammox
Authors: Jia Mingsheng[1,2], Zeng Lin[1,2], Ravikumar Badri Narayan[1,2], Vlaeminck Siegfried[2,3], Carvajal Arroyo Jose Maria[1,4], Boon Nico[1,2], Ganigué Ramon[1,2]

1 Centre for Microbial Ecology and Technology (CMET), Ghent University, Belgium
2 Centre for Advanced Process Technology for Urban Resource Recovery (CAPTURE), Belgium
3 Department of Bioscience Engineering, University of Antwerp, Belgium
4 Colsen B.V., Hulst, The Netherlands

Nitrogen removal from wastewater is crucial for sustainable nitrogen management, one of the major global challenges. Anammox plays a key role in the nitrogen cycle and more resource-efficient nitrogen removal. Despite some molecular evidence of anammox bacteria in thermophilic ecosystems, until recently, all anammox bacteria were exclusively cultivated under mesophilic conditions (<45°C). Consequently, the application of anammox has been limited to mesophilic environments. Long-term cultivation of an active thermophilic anammox culture was recently achieved at 50°C for the first time, which opens the door to develop anammox-based process for treating nitrogen-rich hot wastewaters (e.g. those generated from paper industries and thermophilic sludge digestion) and to better understand the potential role of anammox in thermophilic environments. Here, we aim to comprehensively characterize this novel thermophilic anammox culture. Using a membrane bioreactor and gradually decreasing the sludge retention time (SRT), we achieved over 88% enrichment of planktonic thermophilic anammox culture, belonging to Candidatus Brocadia genus. The thermophilic cultures exhibited stoichiometry typical of mesophilic anammox process. Flow cytometry-based analysis revealed highly active biomass and distinct phenotypic fingerprinting of the microbial community under different conditions. At a record-low SRT of 2.75 days, the observed growth rate (μ) was already 0.36 d^{-1}. Moreover, a maximum specific anammox uptake rate of 5.9±0.3 g NH4-N/g VSS/d was observed (yielding an estimated maximum growth rate (μmax) over 0.7 d^{-1}). These values are the highest ever reported for anammox bacteria. Our results expand the limits of anammox process and highlight the great potential of thermophilic anammox for biotechnological applications.

Session: Biotechnology/Applications
Presenter: Eva Spieck
Title: A novel Nitrospira lineage isolated from activated sludge using elevated temperatures

Authors: Spieck, Eva, Department of Microbiology and Biotechnology, University of Hamburg, Hamburg, Germany; Koch, Hanna, Department of Microbiology, RIBES, Radboud University, Nijmegen, the Netherlands; Lücker, Sebastian, Department of Microbiology, RIBES, Radboud University, Nijmegen, the Netherlands; Keuter, Sabine, Department of Microbiology and Biotechnology, University of Hamburg, Hamburg, Germany

The genus Nitrospira represents the dominant nitrite-oxidizing clade in most wastewater treatment plants (WWTPs) globally, and several Nitrospira strains have been isolated from activated sludge. Usually, the most abundant Nitrospira in these systems belong to lineages I and II and population analyses revealed an extreme inter- and intraspecies diversity of these nitrite-oxidizing bacteria (NOB). This complex synecology of Nitrospira types is potentially driven by niche-defining factors such as oxygen and nitrite availability, and temperature. In this study, activated sludge from the WWTP in Hamburg was screened for novel NOB. Using a pre-enrichment strategy with alternating denitrifying and nitrifying conditions and incubation at elevated temperatures, we isolated a novel Nitrospira species, named N. tepida. This moderately thermophilic species with optimal growth between 37 and 45°C is only distantly related to other Nitrospira and forms a novel lineage VII together with few environmental 16S rRNA gene sequences predominantly detected in thermal wastewater or oxygen-limited systems. Before, other moderately thermophilic Nitrospira were isolated from natural or non-biotechnological systems. Genomic and physiological analyses revealed remarkable differences between N. tepida and two other isolates previously obtained from the same WWTP (N. defluvii, N. lenta), especially within the energy metabolism. The observed catabolic flexibility includes the presence of distinct types of terminal oxidases and the potential of utilizing alternative electron donors like hydrogen. In summary, this novel Nitrospira lineage seems to be well adapted for wastewater treatment processes at elevated temperatures and low aeration, conditions that potentially reduce operational costs of such systems.

Session: Biotechnology/Applications
Presenter: Jonathan Rodriquez
Title: Activity-based ammonia monooxygenase labeling is specific for ammonia oxidizing bacteria and reveals inner membrane localization of the enzyme
Authors: Rodriguez, Jonathan, University of Florida; Choi, EunKyung, University of Florida; Zhao, Jun, University of Florida; and Martens-Habbena, Willm, University of Florida

Ammonia monooxygenase (AMO) homologs mediate ammonia oxidation in all lineages of chemoautotrophic ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB), and complete ammonia oxidizing bacteria (comammox). However, it has not been purified to activity or characterized. The currently predicted structure, localization, and orientation are based on homology to the related particulate methane monooxygenase (pMMO) but has not been experimentally validated in ammonia oxidizers. Here we used an activity-based click-chemistry approach to fluorescently label the AMO in whole cells of AOB and comammox. AMO activity is inhibited with 1,7-octadiyne (1,7OD) followed by a copper-catalyzed azide-alkyne cycloaddition with a fluorescently tagged azide. Using acetylene inactivation of AMO we demonstrate the specificity of the labeling for active AMO and show that this method specifically labels the AMO of Nitrosomonas europaea, Nitrosospira multiformis, and Nitrospira inopinata. Labeled cells were examined by a suite of light, fluorescence, and cryo electron microscopic techniques. These results reveal that AMO in Nitrosomonas europaea is located in invaginations of the cytoplasmic membrane and not facing the periplasmic space. These results support that ammonia and urea transporters may be required for ammonia oxidation in bacteria, irrespective of the orientation of the enzyme. A comprehensive structural model of the ammonia oxidation in Bacteria is presented.

Session: Biotechnology/Applications
Presenter: Michelle M. McKnight
Title: Microbial community analysis reveals a dominance of either commamox Nitrospira or archaea as ammonia oxidizers in freshwater aquarium biofilters

Authors: McKnight, Michelle, M, University of Waterloo, Waterloo, Canada; Neufeld, Josh, D, University of Waterloo, Waterloo, Canada

Nitrification by aquarium biofilters transforms ammonia waste to less toxic nitrate via nitrite. Prior to the discovery of complete ammonia oxidizing (“comammox”) Nitrospira, previous research revealed ammonia-oxidizing archaea (AOA) dominated over ammonia-oxidizing bacteria (AOB) in aquarium biofilters. Here we profiled aquarium biofilter microbial communities and quantified abundances of ammonia oxidizers. Biofilter and water samples were collected from residential and commercial freshwater and saltwater aquaria. We performed 16S rRNA gene sequencing and qPCR on extracted DNA to assess community composition and quantify the abundance of amoA genes, respectively, and high-throughput sequencing of Nitrospira amoA genes. Aquarium biofilter microbial communities were represented consistently by putative heterotrophs of the Proteobacteria and Bacteroides phyla, with distinct profiles associated with freshwater and saltwater biofilters. Comammox Nitrospira were detected in all 38-freshwater aquarium biofilters and were the most abundant ammonia oxidizers in 30, with remaining biofilters dominated by AOA. The AOB were at relatively low abundance in all filters, except for the aquarium with the highest ammonia concentration. For saltwater biofilters, AOA or AOB were differentially abundant, with no comammox Nitrospira detected. Differential distributions of amoA gene sequences from comammox Nitrospira imply niche adaptation based on water chemistry. Additional network analysis of freshwater microbial communities demonstrated significant positive correlations among nitrifiers, and between nitrifiers and other heterotrophic community members, suggesting metabolic and ecological interactions within aquarium biofilter communities. These results demonstrate that comammox Nitrospira play an important and previously overlooked role in biofilter nitrification and that aquaria serve as microcosms for further exploration of nitrifier ecology.

Session: Biotechnology/Applications
Presenter: Liron Friedman
Title: Characterizing nitrogen kinetics under hyper oxygen conditions in soil treatment using model simulation
Authors: Liron Friedman, Columbia University; Hadas Mamane, Tel Aviv University; Kartik Chandran, Columbia University

Characterizing the fate of nitrogen kinetics within wastewater treatment processes for reuse provides key insights into complex reciprocal microbial reactions, potential process performance, and optimized design. However, measuring salient kinetic parameters in the microbial community some parameters, such as growth rates or half saturation rate coefficients, are difficult to extrapolate in a mixed community of various nitrifiers and heterotrophs. Nevertheless, employing model simulators specialized in microbial processes, could reveal the process kinetics and the fate of various species with a good approximation. In this study a novel calculative pipeline is demonstrated to characterize the kinetics of nitrogen when H2O2 was added to intensify infiltration of secondary wastewater effluent by Soil Aquifer Treatment (Friedman et al, 2022). Empirical data from measurements was used to generate a simulated model in BioWin software and simulations results were further analyzed using JMP to evaluate reciprocal interactions related to nitrogen. The findings suggest that although H2O2 addition created local hyper oxygen conditions, simultaneous nitrification and denitrification via nitrite was the dominant nitrogen route and was found to be highly dependent on the fate of organic carbon degradability. Moreover, following community analysis that showed significant dominance for the genus Nitrospira, the simulated model revealed a key role for nitrite in the microbial community kinetics (in control or without H2O2), although nitrite was not detected in the measurements. Revealing the dominant parameters affecting the process enables to focus future research, design more intensive use of existing infrastructure and target the process for minimal environmental footprint.

Session: Biotechnology/Applications
Presenter: Akihiko Terada
Title: Phylogeny, abundance, and function of N_2O-reducing bacteria in an anammox biofilm
Authors: Kohei Oba, Tokyo Univ. Agr. & Technol.; Megumi Kuroiwa, Tokyo Univ. Agr. & Technol.; Akihiko Terada, Tokyo Univ. Agr. & Technol.

Nitrous oxide (N2O) emissions can be mitigated by bacteria harboring N2O reductase. The gene encoding this reductase is classified into two clades. Despite the widespread detection and significance of N2O-reducing bacteria possessing either clade gene, their ecophysiologies require more descriptions for the mitigation of N2O emissions in advanced wastewater treatment. This study, therefore, presents the phylogeny, abundance, and functions of N2O-reducing bacteria in anammox biofilm reactors. For comparison, the two reactors, continuously fed with ammonium and nitrite with or without an external N2O supply, have been operated in parallel for 1200 days. The 16S rRNA gene amplicon sequencing unveiled significant increases in the phylum Chloroflexota in the reactor with N2O supply and in the genus Candidatus Brocadia without N2O supply. Both reactors retained one order of magnitude higher gene abundance of clade II nosZ than clade I nosZ. The hybrid genome assembly and annotation retrieved 42 high- and medium- metagenome-assembled genomes (MAGs) from both reactors. None of the MAGs possessed a complete set of denitrifying genes, suggesting the occurrence of incomplete denitrification. Both reactors harbored clade II nosZ N2O-reducing bacteria, affiliated with Chloroflexota, Ignavibacteria, and Gemmatimonadetes. The genotypes of these MAGs exhibit the lack of either nir gene, nor gene, or both genes. The genotypes of MAGs within the phylum Chloroflexota were diverse, responsible for either N2O consumption or dissimilatory nitrate reduction to ammonia. Collectively, this study illuminates the diverse functions of Chloroflexota as a potential N2O consumer or ammonia producer in an anammox biofilm.

Session: Terrestrial/Soil/Agriculture
Presenter: Gunter V Subbarao
Title: Biological Nitrification Inhibition (BNI) – A nature-based solution for nitrogen problems in agriculture

Authors: Guntur V Subbarao, Masahiro Kishii and Tadashi Yoshihashi, Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

Accelerated nitrifier activity and rapid generation of soil-nitrates in farmlands are the major underlying causes for low nitrogen recovery, nitrogen pollution of -waterbodies (NO_3^--pollution) and -atmosphere (from N_2O, NO emissions), causing ecological damage and inefficiencies in production systems. Nature has evolved multiple strategies to limit nitrifier activity to protect nitrogen from being lost from ecosystems. Buildup of soil organic matter (SOM) levels in natural ecosystems is evidence to that. The ability of certain plant root systems to produce specific antibiotics aimed at soil-nitrifiers, is a plant function termed 'Biological Nitrification Inhibition (BNI)". Production and release of BNIs are functionally linked to ammonium -uptake and -assimilation in root systems. BNI-technology permits delivery of massive amounts of specific antibiotics to soil micro-sites where nitrifying bacteria reside, using root systems as delivery vehicles. Genetic exploitation of BNI function in plant root systems of crops and pastures is a research strategy to develop low-nitrifying, low-N_2O emitting, and nitrogen-efficient sustainable systems. The development of BNI-wheats is presented as a case study to demonstrate the impact of introducing BNI-trait (Lr#n-SA translocation) from a wild-wheat into elite-high-yielding modern wheats, in transforming their BNI-capacity of root systems. Using BNI-isogenic elite-wheat-lines, we have demonstrated that BNIs production in root systems have increased by 3-5 fold, reducing soil-nitrate formation, lowered N_2O emissions and improved nitrogen recovery and grain yields under a wide-range of nitrogen-inputs in field environments. The potential implications from scaling up of BNI-technology in transforming global wheat production systems is the subject of this presentation.

Session: Terrestrial/Soil/Agriculture
Presenter: Willm Martens-Habbena
Title: The role of soil nitrification in soil health, soil carbon retention, and greenhouse gas emissions
Authors: Martens-Habbena, Willm, University of Florida, FL, USA

Nitrification is a central process in the global nitrogen cycle. Over the past two decades the biodiversity of nitrifiers has been substantially extended. In upland soils ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), complete ammonia-oxidizing bacteria (comammox) and nitrite-oxidizing bacteria (NOB) form a complex and diverse network of microbes carrying out this process. In this presentation I will highlight recent findings combining physiological studies on representative microbial strains, soil incubation studies, as well as in situ field studies. The results provide detailed insights into physiological and ecological functions of different ammonia oxidizer lineages in soils. I posit that that AOA and AOB have developed strategies that avoid direct competition for substrates. Akin to the oligotrophic AOA in the global oceans, the most abundant AOA lineages in soils global are slow-growing, ultraoligotrophic ammonia oxidizers. Therefore, oligotrophic AOA in soils and oceans exert strong controls over the reduced N pools in soils globally.

Session: Terrestrial/Soil/Agriculture
Presenter: Soo Hyun Park
Title: Differential response of nitrate-reducing synthetic communities to varying carbon-to-nitrogen ratio

Authors: Park, Soo Hyun, Department of Biological Systems Engineering, University of Nebraska-Lincoln, NE, USA; Song, Bongkeun, Department of Biological Sciences, Virginia Institute of Marine Science, William & Mary, Gloucester Point, Virginia, USA; Song, Hyun-Seob, Department of Biological Systems Engineering, University of Nebraska-Lincoln, NE, USA, Department of Food Science and Technology, Nebraska Food for Health Center, University of Nebraska-Lincoln, NE, USA

Denitrification (DNF) and dissimilatory nitrate reduction to ammonium (DNRA) are two major nitrate-reducing pathways in soil. Both DNF and DNRA bacteria competitively use nitrate as an electron acceptor for the conversion into different nitrogen compounds, i.e., DNF releases a greenhouse gas (nitrous oxide) into the air whereas DNRA retains the nitrogen in the soil as ammonium. Relative abundances of DNF vs. DNRA bacteria are therefore key factors determining the fate of nitrate, which is primarily influenced by carbon-to-nitrogen ratio (CNR), as well as other factors such as redox potential and the generation time of bacteria. This work examines how CNR controls the coexistence and relative abundances of DNF and DNRA bacteria in a soil community. We designed a synthetic community with 588 nitrate-reducing bacterial isolates that was incubated under three CNR conditions (high, medium, and low). The changes in community structures were monitored by 16S metabarcoding and shotgun metagenomic analyses along with measuring nitrate reduction activities. Overall, the community was mainly dominated by Kosakonia, Paenibacillus, and Pseudomonas at the genus level. The relative abundance of DNF (Pseudomonas) over DNRA bacteria (Kosakonia and Paenibacillus) was highest at the medium CNR and then the low and high CNR conditions. Such a nonlinear control of CNR over the relative abundance of DNF and DNRA bacteria revealed in this work provides new insights into their complex interactions in a soil community.

Session: Terrestrial/Soil/Agriculture
Presenter: Soo Hyun Park
Title: Divergent responses of soil nitrifying populations to long-term climate warming
Authors: Li, Hanyan[1], Wang, Baozhan[2], Xia, Lei[2], Zheng, Yue[3], Wang, Dongyu[1], Han, Shun[1], Zhang, Ya[4], Wu, Linwei[5], Liu, Xiaojun[1], Shi, Zheng[1], Wu, Liyou[1], Stahl, David,A.[6], Martens-Habbena, Willm[7], Pan, Chongle[1], Ning, Daliang[1], Zhou, Jizhong[1],*, Qin, Wei[1],*

1 Institute for Environmental Genomics, Department of Plant Biology and Microbiology, School of Biology Sciences, University of Oklahoma, Norman, OK, USA.
2 Department of Microbiology, College of Life Sciences, Nanjing Agricultural University, Nanjing, China.
3 College of the Environment and Ecology, Xiamen University, Xiamen, China.
4 Yokogawa, Houston, TX, USA.
5 College of Urban and Environmental Sciences, Beijing University, Beijing, China.
6 Department of Civil and Environmental Engineering, University of Washington, Seattle, USA.
7 Department of Microbiology and Cell Science, Fort Lauderdale Research and Education Center, University of Florida, Davie, FL, USA.
*Corresponding authors

Nitrification is an essential process in soil nitrogen cycling. Four major lineages of nitrifying microorganisms, the ammonia-oxidizing archaea and bacteria (AOA, AOB), complete ammonia oxidizers (comammox), and canonical nitrite-oxidizing bacteria (NOB) collectively drive the oxidation of the most reduced to the most oxidized forms of nitrogen, thereby controlling the availability and transformation of bioavailable nitrogen in soils. Anthropogenic climate change poses the greatest threats to biodiversity and associated key ecosystem functioning from local to global scales. However, little is known about the effects of long-term climate warming on the community structure and abundance of soil nitrifiers and its ecological consequences for nitrogen cycling. We therefore leveraged a long-term climate change experiment in a grassland ecosystem to investigate the effects of climate warming on soil nitrifying communities over 11 years. We show that the structure and diversity of nitrifying communities are highly sensitive to the long-term experimental warming. A 3 ºC increase in experimental warming significantly enhanced the relative abundance and richness of AOA, while reducing those of AOB, comammox, and canonical NOB, demonstrating the divergent responses of different nitrifier lineages to climate warming. Metagenomic binning yielded 137 AOA and 34 comammox and canonical NOB metagenome-assembled genomes from experimental sites, which enabled us to identify unique genetic capacities associated with the adaptation of specific nitrifier lineages to climate warming and concomitant drought conditions. Our findings make a significant contribution to understanding and predicting the ecological consequences of climate change on the nitrogen cycle in the current and future warming world.

Session: Terrestrial/Soil/Agriculture
Presenter: Andrew Giguere
Title: Application of NH3 and pH optodes to visualize NH3 production and diffusion from fertilizer nitrogen and impacts on nitrification activity

Authors: Merl, Theresa, Aarhus University Centre for Water Technology, Department of Biology, Section for Microbiology; Sedlacek, Christopher J., Division of Microbial Ecology, Center for Microbiology and Environmental System Science, University of Vienna; Pjevac, Petra, Division of Microbial Ecology, Center for Microbiology and Environmental System Science, University of Vienna, Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna; Fuchslueger, Lucia, Division of Terrestrial Ecology, Center for Microbiology and Environmental System Science, University of Vienna; Koren, Klaus, Aarhus University Centre for Water Technology, Department of Biology, Section for Microbiology; Giguere, Andrew T., Division of Microbial Ecology, Center for Microbiology and Environmental System Science, University of Vienna

In agricultural soils, nitrogen fertilizer is typically applied as ammonium salts or urea and undergoes microbially mediated oxidation. However, it remains unclear which concentrations nitrifiers are exposed to in the zones immediately surrounding fertilizer granules and how this affects their activity, as different groups of nitrifiers are adapted to and inhibited by a wide range of ammonia concentrations. To examine these small-scale (<1 cm) changes with high spatiotemporal resolution, we applied a newly developed ammonia and pH optode system to visualize subsurface ammonia production in an agricultural soil from both ammonium chloride and urea hydrolysis. Ammonia was detectable for up to 65 h, diffused up to 2 cm from the fertilizer granules, reaching concentrations >120 ppmv. Spatially informed destructive subsampling revealed that nitrate accumulation was highest closest (0.5-1 cm) to ammonium chloride granules, while nitrite was not detected. In contrast, urea fertilization resulted in nitrite and nitrate accumulation and concentrations were highest furthest (1.5-2 cm) from urea granules. Urea hydrolysis also caused a drastic localized pH increase, while ammonium chloride caused localized increases and decreases in pH. Transcriptional activity of the ammonia-oxidizing bacteria amoA was detected in all subsamples but was higher in the areas furthest from the urea granule, while there were no significant differences from ammonium chloride granules. Ammonia-oxidizing archaea and comammox amoA transcripts were not detectable. This study is the first to directly visualize small-scale changes in ammonia and pH and link these to nitrification activity, demonstrating their potentially significant impacts on nitrifiers.

Session: Terrestrial/Soil/Agriculture
Presenter: Paula Rojas-Pinzon
Title: Biological nitrification inhibitor efficacy depends on compound concentration, microbial community composition and is resilient to short-term soil pH changes.

Authors: Rojas-Pinzon, Paula, A, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Prommer, Judith, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Karbon, Iris, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Madani, Konstanze, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Sedlacek, Christopher, J, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Pjevac, Petra, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Fuchslueger, Lucia, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria; Giguere, Andrew, T, University of Vienna, Centre for Microbiology and Environmental Systems Science, Department for Microbiology and Ecosystem Science, Vienna, Austria.

Up to 70% of the nitrogen (N) fertilizer applied to agricultural soils does not end up in crop biomass. Nitrification contributes to substantial amounts of N loss and to counteract its effects, nitrification inhibition by synthetic and biological compounds is being investigated. Nonetheless, for many biological nitrification inhibitors (BNIs) little is known about their impact on different nitrifier communities, their interaction with soil properties as well as their effective concentrations. We here assessed the effect of short-term soil pH changes on the efficacy of commonly used synthetic inhibitors (DCD, DMPP, and nitrapyrin) and three biological inhibitors (methyl 3(4-hydroxyphenyl) propionate (MHPP), methyl 3(4-hydroxyphenyl) acrylate (MHPA), and limonene) in agricultural soils with contrasting pH and nitrifier community composition. Additionally, the EC50 for the three biological inhibitors, as well as some off-target effects on the microbial community were determined. Differences observed in the efficacy of nitrification inhibitors among soils seem to be driven by a combination of differences in the microbial communities, soil physicochemical properties, and off-target effects. Notably, the efficacy of both synthetic and biological inhibitors was resilient to short-term soil pH changes, and similar compound-specific EC50 was observed in distinct soils. MHPA was determined to be a putative AOB-selective inhibitor, and together with limonene effectively inhibited nitrification at concentrations comparable to the commonly used synthetic inhibitor DCD (between 10 and 20% of the N applied). Our results show that some BNIs could be an effective alternative to SNIs in agricultural soils.

Session: Terrestrial/Soil/Agriculture
Presenter: Ida Peterse
Title: Elucidating acidic ammonia oxidation by “Candidatus Nitrosacidococcus tergens” sp. RJ19

Authors: Peterse, Ida, F, Cluster of Microbiology and Cluster of Ecology and Physiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen; Veraart, Annelies, J, Cluster of Ecology and Physiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen; Jetten, Mike, S. M., Cluster of Microbiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen; Lücker, Sebastian, Cluster of Microbiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen

Ammonia oxidation was assumed to be impossible in highly acidic environments, as protonation to ammonium leads to reduced substrate availability for ammonia-oxidizing microorganisms. Recently, however, different acid-tolerant ammonia-oxidizing microbes have been isolated, including “Candidatus (Ca.) Nitrosacidococcus tergens” that still showed activity at pH 2.5 in continuous culture with ammonium as sole energy substrate. At pH < 6, rather than nitrite, nitric oxide and nitrate were the dominant N-compounds produced. However, the exact physiology of “Ca. Na. tergens” at both acidic and neutral conditions remains uncertain due to chemical instability of intermediates like nitrite and nitric oxide, and the absence of genes like cycA and nirK, which are highly conserved in most other ammonia oxidizers. Here, we investigated the nitrogen metabolism of a “Ca. Na. tergens” enrichment culture under gradual changing pH. Nitrogenous compounds (ammonium, nitrite, nitrate, nitric oxide, and nitrous oxide), bicarbonate consumption and protein content were monitored during slow increase of pH from 2.5 to 7.5. Subsequently, the experiment was conducted in reverse, reducing pH from 7.0 to 2.5. Nitrogen dynamics in both experiments show stoichiometric conversion of ammonia to nitrite at pH ≥ 6, nitrous oxide production peaking between pH 4.0 and 4.5, and chemical production of nitric oxide and nitrate only at pH ≤ 6. Together with transcriptomic data at multiple pH conditions, this data will elucidate the exact mechanisms enabling “Ca. Na. tergens” to perform ammonia oxidation at extremely acidic conditions, and allow for a better understanding of the importance of nitrification in acidic environments.

Session: Terrestrial/Soil/Agriculture
Presenter: Xia Zhu-Barker
Title: Exploring feammox in cultivated wetland soils: A twelve-year incubation study and its implications
Authors: Zhu-Barker, Xia, University of Wisconsin-Madison; University of California-Davis

Feammox (anaerobic ammonium oxidation coupled to iron(III) reduction) in soils is recognized, but the mechanisms and factors controlling this nitrogen transformation pathway remain elusive. To address this, a long-term incubation study was launched in 2011, aiming to 1) test NH4+ oxidation under iron-reducing conditions, 2) scrutinize the impact of different NH4+ concentrations and carbon sources on iron reduction, and 3) evaluate the influence of soil types. Three types of cultivated wetland soils were treated with two nitrogen levels (100 vs. 1000 ug N/kg) and three carbon sources (inorganic C, organic C, or rice residual). In brief, 5g of soil was incubated in 60 ml microcosms with 120% water holding capacity, ensuring anaerobic conditions via sealing and flushing the microcosms with Helium. Throughout the first year, headspace gas samples and soil samples were collected regularly, analyzed for a range of markers including N2O, N2, soluble Fe, Fe(II), Fe(III), DOC, NH4+, and NO3-. First-year results revealed that high NH4+ concentrations hindered the reduction of Fe(III) to Fe(II) and organic matter decomposition, suggesting the absence of Feammox activity. The incubation continued till 2023, with a constant temperature of 22oC maintained in the microcosms. A comprehensive isotopic analysis was carried out, including 13CH4, d2H-CH4, 13CO2, 15N2O, and 15N2 in gas samples, and 13C and 15N in soil samples. This data will elucidate the changes in soil C and N cycles over twelve years, offering critical insights into Feammox processes under diverse environmental conditions.

Session: Terrestrial/Soil/Agriculture
Presenter: Eunkyung Choi
Title: Oligotrophic ammonia oxidation in Nitrosocosmicus sp. R2
Authors: Choi, Eunkyung, Fort Lauderdale Research & Education Center, Department of Microbiology & Cell Science, University of Florida, Davie, FL; Rodriguez, Jonathan, Fort Lauderdale Research & Education Center, Department of Microbiology & Cell Science, University of Florida, Davie, FL; Martens-Habbena, Willm, Fort Lauderdale Research & Education Center, Department of Microbiology & Cell Science, University of Florida, Davie, FL

The first and rate-limiting step of nitrification, the oxidation of ammonia (NH3) to nitrite (NO2$^-$) is carried out by a complex network of chemolithoautotrophic microorganisms including ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and complete ammonia-oxidizing bacteria (comammox). In line with environmental studies, AOA and comammox strains were shown to possess substantially higher ammonia affinities than AOB, suggesting kinetics as important factor of niche differentiation among the major ammonia oxidizer lineages. Intriguingly, the genus Nitrosocosmicus abundant in soils were shown to possess AOB-like low affinities for NH3. Nevertheless, our group enriched Nitrosocosmicus sp. R2 under nutrient limitation from Everglades agricultural soil. Here we report kinetic properties of ammonia oxidation in Nitrosocosmicus sp. R2 with NH3 and urea substrate. Both ammonia-grown and urea-grown Nitrosocosmicus sp. R2 showed high affinities for NH3 (Km = 0.064 ± 0.022 µM NH3 +NH4$^+$), the highest reported ammonia affinity among terrestrial AOA. Surprisingly, Nitrosocosmicus sp. R2 could use urea regardless of whether grown on NH4Cl or urea and exhibited higher urea affinity when grown with urea (Km = 2.058 ± 0.485 µM urea) than NH4Cl (Km = 7.128 ± 1.057 µM urea), indicating that Nitrosocosmicus R2 can adapt their urea utilizing pathways depending on substrate availability. Our results demonstrate significant strain-specific diversity within the genus Nitrosocosmicus, and highlight that oligotrophy is present in soil AOA. These results have important implications for modeling soil nitrification. Further work is needed to cultivate and characterize strains of predominant soil AOA lineages.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Emily Zakem
Title: Understanding and predicting nitrification at global scales
Authors: Zakem, E., Carnegie Institution for Science California, Pasadena, CA

How do we connect nitrification and other nitrogen transformations, regulated at the microscale, to global scale processes? Accurate quantification of these transformations is critical for understanding microbial feedbacks to climate change. In order to predict nitrification in unobserved environments (such as the future), biogeochemical models need broadly applicable parameterizations of nitrogen cycling that are consistent with observations, but not site- or organism-specific. As one path towards this goal, the redox chemistry underlying nitrification and other metabolisms can inform model descriptions of microbial metabolic functional types, reducing the number of free parameters. This approach links microbial activity to the environmental chemical potential in ways that are simple enough to incorporate into global biogeochemical models. The activity and biomass of the metabolic functional types emerge dynamically from ecological interactions. Here, I will show how this approach has complemented observational efforts to increase our understanding and predictions of nitrification in both oxygenated and anoxic marine environments.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Michael Beman
Title: Tropical cyclones accelerate the nitrogen cycle in the ocean's largest oxygen minimum zone

Authors: Beman, Michael, University of California, Merced; Genco, Brandon, M., University of California, Merced; White, Margot, E., University of California, San Diego; Koester, Irina, University of California, San Diego; Vargas, Sonia, M., University of California, Merced; Saunders, Jaclyn, K., University of Georgia; Garcia Maldonado, José, Q., CINVESTAV Mérida; Aluwihare, Lihini, I., University of California, San Diego

Tropical cyclones regularly form above the ocean's largest subsurface oxygen minimum zone (OMZ) in the eastern tropical North Pacific Ocean (ETNP), yet how these powerful storms may affect this biogeochemically important region remains unknown. Here we report multiple effects of a Category 4 hurricane (Bud) that occurred during a research cruise in the ETNP OMZ. Profiles and samples collected beneath Bud's wake captured storm-driven alteration of the upper water column, including elevated particulate organic carbon and chlorophyll concentrations indicative of an active phytoplankton bloom. Comparisons of dissolved oxygen profiles between repeat cruises and additional data demonstrate that the OMZ shoaled by 29-50 m, reaching depths as shallow as 41 m. Coincident with the bloom and OMZ shoaling, nitrogen cycling rates and organisms increased but were compressed within the water column, leading to a shallow area of intense activity. Coupled nitrite oxidation and nitrate reduction occurred at <50 m depth, with the potential for substantial exchange of dissolved gasses and compounds between the ocean and atmosphere. Collectively our data demonstrate that tropical cyclones drive OMZ shoaling, supply fresh organic material to these oxygen-depleted waters, and thereby accelerate nitrogen cycling in the ETNP.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Chris A. Francis
Title: Insights into massive seasonal ammonia-oxidizing archaea blooms in South San Francisco Bay
Authors: Rasmussen, Anna, N., Earth System Science, Stanford University; Francis, Chris, A., Earth System Science & Oceans, Stanford University

San Francisco Bay (SFB) is a nitrogen-rich estuary receiving urban and agricultural runoff and wastewater discharge from nearly 8 million human residents, yet has remained surprisingly resilient to classic symptoms of eutrophication. The largest subembayment of SFB, South Bay, has high ammonia concentrations due to wastewater inputs. Our recent work has revealed that massive, months-long blooms of ammonia-oxidizing archaea (AOA) occur in the autumn (October-December) in the South Bay channel, leading to nitrite accumulation in the well-mixed, oxic water column. We further examined the dynamics of these AOA blooms by measuring nitrification rates, quantifying AOA abundance, and analyzing metagenomic and metatranscriptomic data during the autumn of 2018 and seasonally throughout 2019. Nitrite concentrations reached over 10 µM in the water column in November of 2018, coinciding with peak nitrification rates and AOA abundance. Nitrification rates were correlated with AOA abundance in qPCR data and both increased several orders of magnitude between bloom (autumn) and non-bloom seasons. From bloom samples, we recovered an extremely abundant, high-quality Ca. Nitrosomarinus catalina-like AOA metagenome-assembled genome (MAG) that dominated gene transcription during the bloom. This AOA bloom lineage has a streamlined genome but contains urease genes. We also recovered a putative nitrite-oxidizing bacteria MAG that was of much lower abundance and had low transcriptional activity. This study confirms AOA are not only abundant but also active during these recurring blooms, thus influencing other members of the microbial community and biogeochemical cycling in SFB waters.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Nicky Dotsios
Title: Microbial nitrogen cycling in a seasonally stratified marine lake

Authors: Dotsios, Nicky [1]; Van Kessel, Maartje A.H.J[1]; Venetz, Jessica[1]; Wallenius, Anna J[1]; Nuijten, Guylaine H.L. [1]; Van Helmond, Niels A.G.M. [1,2] ; Lenstra, Wytze K[1,2] ; Zygadlowska, Olga M. [1,2] ; Klomp, Robin[1,2] ; Slomp, Caroline, P. [1,2] ; Jetten, Mike S.M[1]; Lücker, Sebastian[1]

1 Cluster Microbiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen, The Netherlands
2 Department of Earth Sciences, Utrecht University, Utrecht, The Netherlands

The microbial nitrogen (N) cycle is one of the major biogeochemical cycles on Earth. In many coastal ecosystems N cycling is perturbed by anthropogenic activity. Microbial conversions of ammonium, nitrite, and nitrate produce N2, which can ultimately deplete the system of bioavailable N. The seasonally stratified, marine Lake Grevelingen (SW Netherlands) becomes anoxic below the thermocline during summer, which results in a lack of electron acceptors. The anaerobic processes controlling N loss from Lake Grevelingen are not well understood. Although the abundance of aerobic nitrifying microorganisms in oxygen minimum zones is increased, the metabolic pathways to oxidize ammonium under oxygen limiting conditions remains unclear. To investigate N cycling in the water column of Lake Grevelingen, we measured oxygen and nitrogen compounds at high vertical resolution and determined aerobic and anaerobic ammonia and aerobic nitrite-oxidation rates at several depths throughout the year. Furthermore, we analyzed the microbial diversity by 16S rRNA gene amplicon sequencing. Intriguingly, the chemical data showed that nitrite peaked at the oxic-anoxic interface in late summer, which coincided with an increase in the abundance of 16S rRNA genes of aerobic ammonia-oxidizing bacteria belonging to the family Nitrosomonadaceae. In addition, ammonia oxidation was measured at several depths under aerobic conditions. Thus, our study provides valuable insights into the spatiotemporal dynamics and activity of the microbial N cycle network in a eutrophic basin, and thereby contributes to a better understanding of oxygen-limited coastal ecosystems.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Claudia Frey
Title: Oxidative and reductive nitrous oxide production within the Amazon River plume

Authors: Burtscher, Emma, University of Basel, Aquatic and Stable Isotope Biogeochemistry, Department of Environmental Science, Bernoullistr. 30, CH-4056 Basel, Switzerland; Voss, Maren, Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Seestr. 15, D-18119 Rostock, Germany; Lehmann, Moritz, F., University of Basel, Aquatic and Stable Isotope Biogeochemistry, Department of Environmental Science, Bernoullistr. 30, CH-4056 Basel, Switzerland; Frey, Claudia, University of Basel, Aquatic and Stable Isotope Biogeochemistry, Department of Environmental Science, Bernoullistr. 30, CH-4056 Basel, Switzerland

Coastal oceans represent a major source of N2O to the atmosphere. Especially in association with large river inflows, uncertainties remain regarding N2O emissions in association with plume-specific biogeochemical conditions (e.g., high substrate availability). The Amazon River discharges one third of all freshwater into the Atlantic Ocean, and thus far, associated N2O production along the river plume remains largely uncharacterised. The current study investigates the relative importance of different N2O sources (i.e., production by nitrification versus denitrification) in river water, coastal and offshore waters using a combination of stable isotope approaches (e.g., N2O isotopomer profiling and 15N-labelling experiments). Samples were collected during peak river discharge in April/May 2021. Our results revealed a slight oversaturation of N2O concentrations throughout the water column at the river mouth, as well as in the center of the plume. The intramolecular distribution of 15N within the linear N2O molecule, known as site preference (SP), varies between 5 ‰ in bottom waters to 32 ‰ in the upper water column, suggesting that nitrification dominates N2O production in sub-surface waters and denitrification in bottom waters. 15N- label incubation experiments confirm highest reductive N2O production close to the sediment surface, yet at overall relatively low N2O production rates typical for oxygenated marine environments. Based on the data from the Amazon River plume, we conclude that freshwater inputs via large rivers into coastal zones do not necessarily increase N2O production through higher substrate availability. Hence, large river plumes are not always significant contributors to global N2O emissions.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Weiyi Tang
Title: Ammonia oxidation and anammox in the seasonally hypoxic Chesapeake Bay
Authors: Tang, Weiyi; Fortin, Samantha; Intrator, Naomi; Lee, Jenna; Kunes, Moriah;
Jayakumar, Amal; Ward, Bess; Princeton University, Princeton, NJ, USA

Excess nitrogen (N) in coastal waters leads to eutrophication and oxygen depletion, detrimentally impacting the environment. A better understanding of N cycling processes could inform water quality management. As the largest estuary in the United States, Chesapeake Bay experiences seasonal hypoxia in summer with ammonium (NH4+) concentrations reaching 15 µM in bottom waters. We used 15NH4+ tracer incubations to directly measure ammonia oxidation and anammox in surface, middle and bottom waters (oxygenated, oxycline and anoxic respectively), and assess their substrate kinetics and oxygen sensitivities. Ammonia oxidation rates were saturated at middle and bottom depths due to the high ambient NH4+ concentration. The half-saturation constants of ammonia oxidation in surface waters were 0.38-0.75 µM NH4+, higher than those typically found in the open ocean. Ammonia oxidation is also sensitive to oxygen concentration, having half-saturation constants of 1.2-3.0 µM O2, higher than those found in open ocean oxygen minimum zones. The observed half-saturation constants of NH4+ and oxygen for ammonia oxidation in the Chesapeake Bay are distinct from the values observed in the open ocean and can be applied to model nitrification and predict ammonium concentration changes in coastal waters. In addition, anammox rates were mostly below detection but increased up to 5 nmol N2 L^{-1} $d^{-}1$ with the addition of nitrite. Denitrification up to 1700 nmol N2 L^{-1} d^{-1} was the dominant N removal process. The low ambient concentration of nitrite and nitrate may partially be responsible for the relative importance of anammox and denitrification to N loss.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Xin Sun
Title: The explicit representation of diverse denitrifiers in a model resolves observed nitrogen dynamics in marine oxygen minimum zones
Authors: Sun, Xin, Carnegie Institution; Buchanan, Pearse, Carnegie Institution; Zhang, Irene, MIT; Babbin, Andrew, MIT; Zakem, Emily, Carnegie Institution

Denitrification is a multi-step process responsible for fixed nitrogen loss and the production and consumption of N2O, a greenhouse gas and ozone-depleting agent. Denitrification is an anaerobic process that occurs in suboxic and anoxic environments such as marine oxygen minimum zones (OMZs). While denitrification is often considered as a single step in OMZs, metagenomic observations suggest that diverse denitrifiers processing partial or complete denitrification pathways coexist in anoxic seawaters. Here, we represent the diversity of denitrifiers via a theoretical modeling framework, where explicit microbial functional type populations are nested within a marine ecosystem model. We use the free energy associated with the underlying redox reaction to inform the biomass yield of each denitrifier. Our model captures the relative abundances of different denitrification genes, key nitrogen transformation rates, and the co-existence of diverse denitrifying metabolisms observed in OMZs. The results provide an evolutionary perspective for the development of the later denitrification steps and show that considering diverse denitrifiers can affect biogeochemical outcomes. This work shows how the characterization and prediction of biogeochemistry can be informed and tested by the vast amount of publicly available metagenomics datasets.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Mark Trimmer
Title: Production of 15N2 gas from 15NH3 in oxic marine and freshwater sediments
Authors: Trimmer, Mark, Queen Mary University of London

The coupling between nitrification and di-nitrogen gas production is a key step in the nitrogen (N) cycle that recycles ammoniacal-N back to the atmosphere. This key step has been researched widely in both marine and fresh water sediments and is taken to be driven by the distinct microbial processes of aerobic nitrification and the anaerobic processes of denitrification and anammox. In 2021, we argued for a likely direct aerobic oxidation of ammonia to di-nitrogen gas in oxic riverbed sediments that was supported later that year by others working with pure cultures. Here I show how oxic surface sediment layers (~10mm to 15mm) collected in the Irish Sea (70m to 100m water depth) also produce 15N-labelled di-nitrogen gas from 15N-amminia in oxic-slurry incubations. This seemingly aerobic production of 15N-labelled di-nitrogen gas runs in parallel to the expected production of 15N-nitrate through nitrification. Further, comparison of these recent measurements of oxic di-nitrogen gas production with our earlier (2009) measurements of anoxic di-nitrogen gas production shows the two modes gas production to be potentially comparable. Strikingly, whereas the riverbed sediments harbouring the seemingly aerobic production of di-nitrogen gas were well-irrigated, oxic gravels and sands, these marine sediments are muddy, with typical diffusion driven oxygen exchange. We are working to characterise the genes that may be involved in the direct aerobic oxidation of ammonia to di-nitrogen gas in both marine and freshwater sediments.

Session: Global Nitrogen Cycling in a Warming World
Presenter: Mhlangabezi Mdutyana
Title: Nitrification in the Southern Ocean: controls, kinetics, and biogeochemical implications

Authors: Mdutyana, Mhlangabezi, University of Cape Town, Department of Oceanography, Rondebosch, Cape Town, South Africa; Ward, Bess, B., Princeton University, Geosciences Department, Princeton, NJ, USA and Fawcett, Sarah, E., University of Cape Town, Department of Oceanography, Rondebosch, Cape Town, South Africa

Across the wintertime Southern Ocean, nitrification is the dominant biological process acting on mixed-layer nitrate, producing enough newly-nitrified nitrate to support all phytoplankton growth in winter and some in the following spring. We have shown that the two steps of nitrification are decoupled in this region, with nitrite oxidation emerging as rate-limiting. Nitrifiers appear to have a higher affinity for ammonium (Km=28-137 nM) than nitrite (Km=134-403 nM), with nitrite oxidizers apparently requiring a minimum nitrite concentration of ≥115 nM to produce nitrate. This latter trait may help to explain the Southern Ocean's perennially elevated mixed-layer nitrite concentrations (150-200 nM). Curiously, despite active ammonium uptake and oxidation, ammonium concentrations remain elevated (0.5-1 µM) throughout the winter mixed layer of the polar Southern Ocean. Ammonia oxidation is apparently saturated at low substrate concentration, with the maximum rate of ammonia oxidation remaining near-constant regardless of ammonium concentration, temperature, or light. This finding, along with the results of laboratory experiments conducted by others, led us to hypothesize that ammonia oxidation may be sensitive to iron availability. To test this hypothesis, we recently conducted iron addition experiments using seawater collected at the base of the winter mixed layer across the Southern Ocean. Our preliminary results suggest a modest response of ammonia oxidation rates to iron addition and that the in-situ ammonia oxidizers can use the exogenous siderophore, desferrioxamine B. We also investigated changes in ammonia oxidizer community composition in response to iron; our rate data will be interpreted in the context of these results.

Session:	Symbiosis/Interactions
Presenter:	Jana Milucka
Title:	Diversity and distribution of denitrifying respiratory symbionts
Authors:	Milucka, Jana, Max Planck Institute for Marine Microbiology, Bremen, Germany

Denitrification, dissimilatory reduction of nitrate to N2, is a metabolic process of anaerobic respiration, and a key mechanism responsible for removal and loss of nitrogen from natural and man-made ecosystems. This functional trait is widespread among prokaryotes, such as bacteria and archaea, but also some unicellular eukaryotes, including foraminifera, fungi and ciliates, are capable of denitrification. In contrast to prokaryotic denitrifiers, very little is known about the distribution, origin and molecular mechanisms behind eukaryotic denitrification. We recently discovered that in an anaerobic ciliate of the Plagiopylea class, denitrification is performed by its bacterial endosymbiont. The bacterium, ‘Candidatus Azoamicus ciliaticola’ transfers energy obtained from denitrification, in the form of ATP, to its host. This represents a new, ‘respiratory’- type symbiosis that is analogous to mitochondria, eukaryotic energy-providing organelles, which too derived from a free-living bacterial ancestor. In my talk I will present our recent findings regarding the distribution and diversity of denitrifying symbionts. Our data show that Azoamicus-like respiratory endosymbionts broadly occur in lake, marine and groundwater ecosystems. Surprisingly, the symbiont 16S rRNA sequences are most commonly retrieved from wastewater treatment plants, suggesting that such man-made ecosystems represent particularly favorable environment for this symbiosis. The newly recovered complete metagenome-assembled genomes (MAGs) reveal an unforeseen taxonomic diversity of denitrifying endosymbionts, and a surprising plasticity of their genome composition that allows for environment-specific adaptations.

Session: Symbiosis/Interactions
Presenter: Kateryna Zhalnina
Title: Chemoecology of Plant-Archaea Interactions via Linkages Between Metabolites, Genes, and Nutrient Cycling
Authors: Wipf, Edi, M-L, LBNL; Golini, Amber, LBNL; Bowen, Benjamin, LBNL; Northen, Trent, LBNL; Zhalnina, Kateryna, LBNL

Archaea, ancient microorganisms widely distributed in diverse environments, have received limited attention regarding their interactions with other organisms, particularly eukaryotes like plants. Ammonia-oxidizing archaea (AOA) are abundant in the rhizosphere of different plant species, where they play a crucial role in nitrogen transformations. However, the effects of AOA on plant growth and metabolite chemistry, as well as the dynamic interplay between AOA, plants, and their surrounding environment, remain poorly understood. To address this knowledge gap, we conducted a study using controlled sterile fabricated ecosystems, investigating the interaction between one of the most abundant soil AOA (Nitrososphaera viennensis) and the bioenergy crop switchgrass to identify the feedback mechanisms between plant exudates and AOA nitrification activity. Through the integration of biochemistry, metabolomics, and transcriptomics, we characterized the molecular interplay between switchgrass, archaea, and their environment. Our findings revealed that AOA nitrification was influenced by plant age and root exudate profiles. At the early stages of plant growth, AOA activity was stimulated, while nitrification was fully inhibited during later stages of active plant growth. Furthermore, the presence of AOA significantly impacted plant exudation, leading to an increased production of various molecules in the rhizosphere, including alkaloids, amino acids, peptides, and quaternary amines. Notably, metabolites overproduced by plants during stages of complete AOA inhibition demonstrated promising nitrification inhibitors from alkaloids, polyketides, and terpenoids. Additionally, we explored the expression of archaeal genes in response to different plant exudates, providing further insights into the intricate interactions and nutrient flow between plants and archaea in the rhizosphere.

Session: Symbiosis/Interactions
Presenter: Lilly Blume
Title: Ammonia-oxidizing archaea associated with harmful algal blooms in the York River estuary
Authors: Blume, Lilly, T, Virginia Institute of Marine Science; Song, Bongkeun, Virginia Institute of Marine Science; Reece, Kimberly, S, Virginia Institute of Marine Science

Ammonia-oxidizing archaea (AOA) are ubiquitous in aquatic ecosystems. They form part of the nitrifier community, in which they oxidize ammonium (NH4+) to nitrite (NO2-). AOA may primarily impact the nitrogen cycle by influencing inorganic nitrogen (N) species composition and availability. In a 2020 metagenomic study assessing interactions between summer blooms of the harmful dinoflagellates Margalefidinium polykrikoides and Alexandrium monilatum, and microbial communities in the York River Estuary of Chesapeake Bay, unexpectedly high abundances of AOA Nitrosopumilales were found, which exceeded those previously reported. We investigated the association between AOA and these harmful blooms that are common in this region during the summer by conducting a metabarcoding analysis of the 16S and 18S rRNA genes and quantitative PCR (qPCR) of ammonia-monooxygenase (amoA) and 16S rRNA genes. We found that Nitrosopumilales were absent in pre-bloom conditions but became a dominant taxon in the transition period (between M. polykrikoides termination and A. monilatum onset) as well as during the post-bloom period. The relative abundance of AOA was positively correlated with NO2- concentrations, which peaked post-bloom. This may indicate nitrification activity is varying with the 2020 summer bloom succession. Further investigation is needed to better understand the contribution of AOA to bloom initiation and termination, their role in the microbial loop (e.g., connection to heterotrophic bacteria), and the estuarine nitrogen cycle.

Session: Symbiosis/Interactions
Presenter: Katharina Kitzinger
Title: Metabolic interactions and genome evolution in marine AOA - sponge symbioses

Authors: Kitzinger, Katharina, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Glasl, Bettina, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Moeller, Florian, U., Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Herbold, Craig, W., Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria & University of Canterbury, New Zealand; Rix, Laura, University of Queensland, Australia & Australian Institute of Marine Science, Australia; Engelberts, Pam, J., University of Queensland, Australia; Luter, Heidi, Australian Institute of Marine Science, Australia; Damjanovic, Katarina, Australian Institute of Marine Science, Australia; Schintlmeister, Arno, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Mooshammer, Maria, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Motti, Cherie, Australian Institute of Marine Science, Australia; Robbins, Steven, J., University of Queensland, Australia; Behnam, Faris, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Watzka, Margarete, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Schweder, Thomas, Institute of Marine Biotechnology e.V, Germany & University of Greifswald, Germany; Albertsen, Mads, Aalborg University, Aalborg, Denmark; Richter, Andreas, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria; Bourne, David, G., Australian Institute of Marine Science, Australia & James Cook University, Australia; Webster, Nicole, S., University of Queensland, Australia & Australian Institute of Marine Science, Australia & Australian Antarctic Division, Australia; Wagner, Michael, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria

Marine sponges are one of the few examples of animals hosting ammonia oxidizing symbionts, which are considered essential for removing nitrogenous waste products from the host tissue. However, most studies to date rely on metabolic modeling, with few insights into the functioning and interaction partners of ammonia oxidizing archaea (AOA) in the sponge holobiont. Here, we characterize contrasting AOA symbionts of two tropical sponges by combining visualization, activity measurements, and -omics techniques. We show experimentally that in the sponge Ianthella basta, a tight metabolic network consisting of its AOA and gammaproteobacterial symbionts together recycles and oxidizes taurine, a ubiquitously occurring sulfonate metabolite in marine sponges. While the I. basta AOA symbiont shows genomic features typical for sponge-associated AOA with a large genome (ca. 2 Mbp), we detected a highly unusual AOA group in a co-occurring sponge genus. These AOA have strongly streamlined genomes (ca. 0.9 Mbp), representing the smallest known AOA genomes to date, with features typical for endosymbionts: low GC-content and pseudogenization, and high coding density. Nevertheless, key genes for ammonia oxidation, energy flow, carbon fixation, vitamin, and amino acid biosynthesis are conserved and actively transcribed. Accordingly, we measured active nitrification in the sponge holobiont. These highly reduced genomes can serve as minimal genome proxies for AOA due to their fully functional core metabolism and may help to elucidate unresolved steps of the archaeal ammonia oxidation pathway. Together, our results shed light on AOA metabolic interactions and indicate that some AOA live in unexpectedly close relationship with their host.

Session: Symbiosis/Interactions
Presenter: Man-Young Jung
Title: Competitive inhibition of ammonia-oxidizing microorganisms in co-culture systems at high and low ammonia concentrations
Authors: Seongwook Kim, Jeju National University; Man-Young Jung, Jeju National University

In natural ecosystems, ammonia-oxidizing microorganisms AOM [AOMs; ammonia-oxidizing archaea (AOA), bacteria (AOB), and complete ammonia oxidizers (Comammox)], strains commonly coexist and compete with each other for NH3 as the sole energy and N source. However, their clear niche differentiation, adaptation, and survival strategies are not still established yet. In this study, we revealed that the total NH3 oxidation rate and the changes in cell yield during NH3 oxidation were decreased in a co-culture system where high-affinity type AOA (Nitrosarchaeum koreense MY1) and low-affinity type AOB (Nitrosomonas europaea ATCC25978) strains were incubated together at low ammonia concentration. Furthermore, at growing low-affinity type AOA (Nitrosocosmicus oleophilus MY3) and AOB (N. europaea ATCC25978) strains together with high ammonia concentration, NH3 oxidation rate and cell yields of AOM were also decreased. Consequently, our results indicate that previously reported substrate oxidation affinity and activity of AOMs in the ecological system are strongly influenced by inhibition during competition between different strains for their common substrate NH3. Therefore, we could provide significant insights into what may fundamentally change the established theory on AOMs in microbial ecology.

Session: Symbiosis/Interactions
Presenter: Mara Walters
Title: Microplastics alter nitrification activity in the ammonia-oxidizing bacterium Nitrosospira sp. AV

Authors: Walters, Mara F, Virginia Institute of Marine Science/William & Mary; Song, Bongkeun, Virginia Institute of Marine Science/William & Mary; Seeley, Meredith E, National Institute of Standards and Technology, Hawaii

Microplastics are ubiquitous in the environment and their toxicity has been reported in a diverse array of organisms. A previous study has shown that microplastics can affect the nitrification activities of sedimentary microbial communities. However, it is unclear which nitrifying taxa are being negatively impacted by exposure to microplastics of different polymer types. Additionally, it is uncertain whether UV-weathered microplastics will affect nitrifying microbes differently than non-UV-weathered microplastics. It is also important to consider the role that leachates of plastic additive chemicals may play in toxicity. To begin to answer these questions, we conducted an exposure experiment with cultures of the ammonia-oxidizing bacterium Nitrosospira sp. AV over the course of four days. Untreated or UV-weathered microplastics composed of polyethylene (PE), polylactic acid (PLA), polyurethane foam (PUF), or polyvinyl chloride (PVC) were added to cultures at a concentration of 1 mg/mL. Changes in bacterial cell numbers and concentrations of nitrite and nitrate were measured to determine impacts on bacterial growth and nitrification rates, respectively. Pyrolysis coupled with GC/MS (PyGC/MS) was utilized to determine the additive composition of the microplastics before and after UV-weathering. Our results showed that only untreated PVC microplastics inhibited the nitrification activity of Nitrosospira sp. AV during the entire experiment, whereas UV-weathered PVC microplastics did not. UV oxidation of an abundant flame retardant additive may have mediated the difference in toxicity. This study demonstrates that microplastics can inhibit the nitrification activity of ammonia-oxidizing bacteria and suggests that UV exposure and additive composition can impact toxicity.

Session: Symbiosis/Interactions
Presenter: Maximilian Dreer
Title: Ammonia oxidizing archaea cultivated from human skin
Authors: Dreer, Maximilian, University of Vienna; Yüksel, Ülkü, University of Vienna; Hodgskiss, Logan, University of Vienna; Kerou, Melina, University of Vienna; Schleper, Christa, University of Vienna

Ammonia oxidizing archaea (AOA) play a crucial role in the global nitrogen cycle by converting ammonia to nitrite. They are part of the nitrifying communities in marine, freshwater and terrestrial ecosystems and outnumber ammonia oxidizing bacteria in many environments. Interestingly, only AOA but not AOB or comammox bacteria have been discovered as part of the human skin microbiome using molecular techniques. However, the significance of these finding has also been disputed. Here we present the cultivation of AOA from three different human individuals. Each enrichment contains a distinct and novel AOA species, belonging to the genus Nitrosocosmicus, members of which are found in diverse terrestrial environments. The organisms oxidize ammonia to nitrite stoichiometrically which coincides with cell growth. Light microscopy and scanning electron microscopy images show their spherical cell morphology as well as their preference to form aggregates like other representatives of this genus. Comparative genomics shows their ability to produce extracellular polymeric substances (EPS) and reveals metabolic features unique to the skin-derived strains, such as e.g. the potential to oxidize compounds encountered on the skin surface. The potential beneficial role of AOA on human skin is intriguing considering that nitric oxide (NO), an important signaling molecule involved in diverse processes such as neuronal signaling, immune response and wound healing in humans, is produced during the ammonia oxidation process. While the influence of AOA on human health remains to be elucidated, we unequivocally show that they are present and culturable from human skin.

Session: Functional Omics
Presenter: Alyson Santoro
Title: Genomic and metabolomic approaches to understanding the ecology of marine nitrite oxidizers
Authors: Alyson Santoro Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA

The connections between “omics” and the cycling of elements in the ocean are not always obvious or direct. I will discuss recent research by our group attempting to connect various ‘omic approaches, including genomics, proteomics and metabolomics of marine nitrite oxidizers with geochemical rates and the distribution of various elements in the ocean. I will show that nitrifiers, in particular nitrite oxidizers, can impact the cycles of trace metals in carbon in addition to their well-known role in nitrogen cycling. In this way, they form an integral part of the deep-ocean food web.

Session:	Functional Omics
Presenter:	Xuefeng (Nick) Peng
Title:	The ecology of active nitrifiers in the eastern tropical North Pacific oxygen minimum zone
Authors:	Peng, Xuefeng, School of Earth, Ocean and Environment, University of South Carolina

Nitrification plays a pivotal role in remineralization in the ocean. In oxygen minimum zones, nitrifiers are often positioned by a sharp oxycline to directly interact with denitrifying and anammox bacteria, as well as cyanobacteria responsible for cryptic oxygen cycling. We used metagenomics and metatranscriptomics to investigate the diversity and activities of ammonia- and nitrite-oxidizing organisms in the eastern tropical North Pacific. At offshore stations, Ca. Nitrosopelagicus brevis were the most active ammonia oxidizers whereas at the coastal station, Nitrosopumilus sp. SW and Nitrosomarinus catalina were the most active members of the ammonia-oxidizing community. We identified an ancestral clade of archaeal amoA genes that were only found at offshore stations. The expression of amoA genes displayed a subsurface maximum in the upper oxycline at oxygen concentrations < 5µM (around 90m), consistent with previously measured depth profiles of ammonia oxidation rates. A large clade of amoA genes closely related to uncultivated Nitrosopelagicus were active only at the lower oxycline at 900m, where they might play a key role in dark carbon fixation. We reconstructed the genomes of seven different Nitrospina species, all of which showed a subsurface maximum of relative abundance and activity at the oxic-anoxic interface (~120m). The most abundant and active Nitrospina species showed low activity at the secondary nitrite maximum (250m) where in situ oxygen concentrations were below detection (10nM), but all the other six Nitrospina species were the most active at 250m. Our findings suggest distinct lineages of Nitrospina adapted to the oxygen deficient parts of the ocean.

Session: Functional Omics
Presenter: Bongkeun Song
Title: Metaproteogenomic investigation of sedimentary nitrogen cycling communities responding to seawater intrusion in the Nakdong River, South Korea
Authors: Song, Bongkeun, Virginia Institute of Marine Science; Wilson, Stephanie, Smithsonian Environmental Research Center; An, Soonmo, Pusan National University
Huang, Yan, Pusan National University; Tobias, Craig, University of Connecticut; Stegen, James, Pacific Northwest National Laboratory; Lipton, Mary, Pacific Northwest National Laboratory
Tolic, Nikola, Pacific Northwest National Laboratory; Nicora, Carrrie, Pacific Northwest National Laboratory

Estuaries are important habitats of diverse biotic communities with high productivity. With the increase of urbanization and land use changes, estuaries have experienced various hydrologic alterations. Estuarine dams are a major anthropogenic disturbance to natural ecosystems converting a brackish flow system to a freshwater reservoir. The Nakdong River in South Korea is an example of the ecosystems heavily impacted by an estuarine dam. In 1987, the Nakdong River Dam was constructed at the mouth of the river to stop tidal seawater exchange and protect freshwater resources. The alteration of flow regimes resulted in consistent seasonal eutrophication over the past 30 years. The Korean government recently developed an innovative plan of water management with controlled openings of the dam gates to restore the natural ecosystem. This system provides an unprecedented opportunity to evaluate the impacts of seawater intrusion on benthic nitrogen cycling communities. Sediment samples collected before and after the dam gate opening in 2019 and 2020 were used to examine the changes in abundance, composition and expression of the nitrogen cycling communities using metagenomic and metaproteomic analyses. A significant reduction of the gene abundance in nitrification and denitrification was observed while the DNRA genes were increased with seawater intrusion due to the dam gate opening. Metaproteomic analysis showed differential expression of denitrification genes in the sediments associated with seawater intrusion. Denitrification rates were temporally reduced and matched with metaproteomic data. Thus, this multi-omics study showed the significant alternation of nitrogen cycling processes due to seawater intrusion in the Nakdong River.

Session: Functional Omics
Presenter: Juliet Johnston
Title: Dissolved Oxygen’s impact on nitrifying bacteria transcriptomic expression in a full-scale attached growth wastewater treatment system
Authors: Johnston, Juliet, T, Georgia Institute of Technology; Vilardi, Katherine, Northeastern University; Cotto, Irmarie, University of Washington; Sudarshan, Ashwin, Georgia Institute of Technology; Gabrielli, Marco, Polytechnical University of Milan; Klaus, Stephanie, Hampton Roads Sanitation District; Bachman, Megan, Hampton Roads Sanitation District; Parsons, Mike, Hampton Roads Sanitation District; Wilson, Christopher, Hampton Roads Sanitation District; Bott, Charles, Hampton Roads Sanitation District; Pinto, Ameet, Georgia Institute of Technology

We recently reported on the co-occurrence and cooperation of aerobic and anaerobic nitrifying bacteria in a full-scale attached growth systems for nitrogen removal from wastewater. While canonical ammonia oxidizing bacteria were present, aerobic ammonia oxidation was primarily driven by complete ammonia oxidizing (comammox) bacteria within the Candidatus Nitrospira nitrosa cluster. Further, Brocadia-like anaerobic ammonia oxidizing (anammox) bacteria leverage the lack of oxygen in dense attached growth biofilms to remove nitrite and residual ammonia further driving nitrogen loss. While aerobic and anaerobic ammonia removal was measured in the full-scale reactors and in batch assays, the precise mechanism for nitrite availability for anammox bacteria is unclear. Specifically, nitrite for anaerobic ammonia oxidation could either be made available by (1) partial nitrification of ammonia by comammox bacteria or canonical ammonia oxidizing bacteria, (2) partial denitrification of nitrate by denitrifying bacteria, or (3) nitrite comproprotionation by comammox or other novel bacteria. Identifying the precise mechanism for nitrite availability is crucial for optimizing nitrogen loss via anaerobic ammonia removal. To better understand microbial processes governing nitrite production, we investigated the changes in transcriptional profiles of the microbial community in this attached growth system across several dissolved oxygen setpoints. We quantified changes in the expression of nitrifying bacteria, and changes in the bulk community's protein synthesis potential by performing RT-qPCR and amplicon sequencing of genes involved in all known aerobic and anaerobic biotransformation’s of ammonia, nitrite, and nitrate. These analyses will be complemented with meta-transcriptomics to further determine pathways regulating nitrite availability.

Session: Functional Omics
Presenter: Graeme Nicol
Title: Identifying novel virus families infecting nitrifying archaea and bacteria in soil
Authors: Lee, Sungeun, University of Lyon; Hazard, Christina, University of Lyon;Nicol, Graeme, W., University of Lyon

While the complexity of prokaryote communities in soil is well understood, there remains a paucity of studies characterising the impact of viruses on hosts, biogeochemistry and rates of ecosystem processes. Microbially-mediated nitrification, central to nitrogen fertiliser losses in soil, is performed by taxonomically and functionally restricted groups of autotrophic organisms and has led them to become established model groups for linking diversity, evolution, ecophysiology and function in soil. However, the diversity and impact of virus infection on these organisms are unknown. To identify active interactions between nitrifiers and viruses, the transfer of assimilated carbon from autotrophic prokaryotes to viruses was examined using DNA stable isotope probing (DNA-SIP) coupled with metagenomic analysis. Combining with a novel hybrid analysis using GC mol% fractionation enabled identification of novel virus families infecting ammonia oxidising archaea (AOA). However, to characterise those also infecting ammonia- and nitrite oxidising bacteria, a second approach used filtration of virus-like particles from nitrifying soil microcosms after differential inhibition to alleviate competition and increase abundance of viruses infecting non-inhibited groups. This dramatically increased the recovery of high-quality virus genomes, with 225 from those infecting nitrifiers, including 69 complete or near-complete genomes ranging in size between 34 and 212 kb, all representing novel virus families. Soil AOA viruses did not contain auxiliary genes associated with ammonia oxidation but were enriched in those implicated in copper sequestration. Infection of nitrifiers is a dynamic process suggesting that understanding virus interactions during nitrification may inform approaches for augmenting activity and associated nitrogen losses.

Session: Functional Omics
Presenter: Bradley Tolar
Title: Environmental changes impact growth, transcription, and protein production of a low-salinity ammonia-oxidizing archaeon

Authors: Tolar, Bradley B., Department of Biology and Marine Biology, University of North Carolina Wilmington; Francis, Christopher A., Departments of Earth System Science & Oceans, Stanford University

Thaumarchaeota are globally distributed, ammonia-oxidizing archaea (AOA) that persist in terrestrial and aquatic environments across a broad range of temperatures, salinities, and pH. These AOA play a key role in the global nitrogen cycle, catalyzing the first and rate-limiting step of nitrification. Though an increasing number of cultivated AOA exist, most of our knowledge on physiological preferences and limitations come from molecular surveys; these studies suggest that AOA tend to prefer habitats with reduced light, temperature, and pH. To directly investigate how environmental factors impact Thaumarchaeota growth and metabolism, we manipulated stable enrichment cultures of “Candidatus Nitrosarchaeum limnium” – the low-salinity “ecotype” found in rivers, lakes, and estuaries worldwide – and collected samples for proteomic and transcriptomic analyses. We subjected “Ca. N. limnium” to changes in temperature, salinity, pH, light, and inorganic nitrogen concentration under environmentally-relevant conditions. Increased temperature and ammonia concentrations, and decreased pH, enhanced growth rates of “Ca. N. limnium”, while lowest growth rates occurred with decreased temperature and light exposure. Proteomics results show an increase in cell exterior proteins under elevated temperature, as well as many stress response and unknown proteins. Preliminary data suggests that higher temperature led to a significantly different transcriptome from “Ca. N. limnium”, and transcripts recovered from increased or decreased salinity treatments were distinct. Both datasets indicate that light exposure showed the most unique protein and transcript profiles. These results will be used to determine genes involved in physiological stress responses in “Ca. N. limnium” and a general characterization of Thaumarchaeota responses to environmental change.

Session: Functional Omics
Presenter: Logan Hodgskiss
Title: Filling gaps in the metabolism of ammonia oxidizing archaea using comparative and functional genomics
Authors: Hodgskiss, Logan, H., University of Vienna; Kerou, Melina, University of Vienna; Schleper, Christa, University of Vienna

Nitrification has been established as a fundamental process across a huge variety of oxic ecosystems. The first step of this process, the oxidation of ammonia to nitrite, is carried out by both bacteria and archaea through distinct yet mostly unresolved pathways. This is particularly true for the highly abundant AOA. On a genomics level, AOA share some similarities to their bacterial counterparts with respect to ammonia oxidation, but contribute to the fixation of carbon by their unique and highly efficient 3-hydroxybutyrate/4-hydroxypropionate pathway. Even so, the flow of energy and carbon from nitrification and carbon fixation to the primary cellular building blocks has remained unclear. To identify essential components and acquire a deeper understanding of AOA metabolism, we have therefore used omics techniques in combination with other comparative and modelling tools. The identification of three additional subunits of the ammonia monooxygenase in AOA was possible through a combination of comparative biochemical and functional genomic analyses of different AOA strains showing that broader approaches can help elucidate gaps in central metabolic modules (Hodgskiss et al. ISME J. 2023). Similarly, key positions have been identified in the central carbon metabolism that link carbon fixation, the TCA cycle, and gluconeogenesis in AOA through functional genomics and proteomics guided by an experimental design based on a thermodynamic model of AOA metabolism (Hodgskiss et al. in preparation). The presented work will also include hypotheses of further crucial metabolic features in AOA (i.e., electron flow) that can be made by equally combining functional and comparative genomic analyses.

Session: Functional Omics
Presenter: Petra Pjevac
Title: Distinct physiological functions of NXR homologues in Nitrospira moscoviensis
Authors: Pjevac, Petra, University of Vienna, Centre for Microbiology and Environmental System Science (CeMESS), Division of Microbial Ecology (DoME), Vienna, Austria; Socolov, Alisa, University of Vienna, Centre for Microbiology and Environmental System Science (CeMESS), Division of Microbial Ecology (DoME), Vienna, Austria; Daims, Holger, University of Vienna, Centre for Microbiology and Environmental System Science (CeMESS), Division of Microbial Ecology (DoME), Vienna, Austria

Nitrite-oxidizing bacteria of the genus Nitrospira are key players in the global nitrogen cycle. Several Nitrospira species also grow by oxidizing alternative substrates, like formate, hydrogen and ammonia (comammox). Some Nitrospira also oxidize formate or hydrogen using nitrate as an alternative electron acceptor. Interestingly, Nitrospira do not encode any canonical nitrate reductases (NARs), but often multiple copies of the periplasmic nitrate oxidoreductase (NXR). N. moscoviensis encodes four genomically co-localized homologues of the α and β subunits (nxrA and nxrB) of NXR, as well as three soluble and two putative, membrane anchored copies of the γ subunit (nxrC), neither of which is co-localized with the nxrAB. The proteins encoded by the four nxrAB loci are highly conserved, with all NxrB being almost identical (>99.5% AA identity), while the NxrA display a slightly higher level of sequence variance (96.2-98.5% AA identity). Despite the high level of protein sequence conservation, nxrAB genes are highly differentially transcribed under different growth conditions. During aerobic growth on nitrite, a single nxrAB homologue is highly transcribed. However, when N. moscoviensis grows aerobically by oxidizing hydrogen or formate, the aforementioned nxrAB locus is transcriptionally nearly silenced. In turn, the transcription of another nxrAB locus is strongly and significantly upregulated. This locus is also highly transcribed when nitrate reduction occurs. Thus, different NXR homologues likely have distinct metabolic functions. These results call in question the validity of Nitrospira nxr gene transcription as functional and activity markers for nitrite-oxidizing members of this widely distributed, but mostly uncultured, genus.

Session: Functional Omics
Presenter: Rui Zhao
Title: A novel bacterial phylum involved in nitrite consumption in marine sediments
Authors: Zhao, Rui, Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA; Babbin, Andrew, R, Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA

Nitrite-oxidizing bacteria (NOB) are important nitrifiers whose activity is critical in regulating the availability of nitrite and also the primary production in ecosystems. In marine oxic sediments where ammonia-oxidizing archaea (AOA) and NOB catalyze the oxidation of ammonium to nitrate, the observed AOA:NOB abundance ratios are significantly higher than the theoretical ratio (3:1) predicted from the energetic perspective, indicating that some NOBs are yet to be discovered. Here we report a new bacterial phylum Candidatus Sedimentinitrospinota, members of which are abundant and widespread in oligotrophic sediments. Ca. Sedimentinitrospinota currently includes three metagenome-assembled genomes (MAGs) from Arctic sediments and three from the Mariana Trench, which represent two different species within the same genus (Candidatus Sedimentinitrospina). Ca. Sedimentinitrospinota have the functional potential of both oxidizing (nitrite oxidoreductase (NXR)) and reducing (copper-containing nitrite reductase (NirK)) nitrite, in addition to other accessary functions (e.g., urea hydrolysis and thiosulfate reduction). Rapid biological nitrite consumption and minor nitrate production are observed in lab enrichments in which nitrite is supplied. Urea addition remarkably accelerates the nitrite consumption rate. While one species (Ca. Sedimentinitrospina aerophilis) is confined in the oxic zone, the other (Ca. Sedimentinitrospina aerotolerans) can thrive in anoxic sediments and may use thiosulfate as the alternative electron acceptor in redox reactions. Counting Ca. Sedimentinitrospinota as NOB can resolve the apparent abundance mismatch between AOA and NOB in marine oxic sediments. Our findings suggest that Ca. Sedimentinitrospinota is among the previously overlooked NOB, whose activity may exert a critical control on the nitrite budget in a wide range of marine sediments.

Session:	Closing session Plenary
Presenter:	Sukhwan Yoon
Title:	Quest for deciphering environmental N2O sinks – ecophysiology of high-affinity N2O reduction
Authors:	Yoon, Sukhwan, Department of Civil and Environmental Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

Since the first reports of the genomic and physiological evidence of the novel *nosZ* clade, now termed *nosZ* clade II, microbial reduction of nitrous oxide (N_2O) has attracted many in search of environmental technology for mitigation of emission of this potent greenhouse gas. Beginning with the breakthrough discovery revealing that certain subgroups of clade II nosZ-possessing organisms have a distinguished ability to consume submicromolar N_2O for utilization as the electron acceptor, we, the past and present members of Environmental Microbiology Laboratory (EML) at KAIST, have made substantial contributions in advancing the physiological and genomic understanding of N_2O reduction, especially pertaining to biological nitrogen removal (BNR) processes in the wastewater sector and nitrogen management of agricultural soils. We have verified, via two disparate avenues of approach, that the clade II *nosZ* dominate both gene and transcript pools of *nosZ* in activated sludge microbiomes. In a subsequent study combining culture-based experiments with computational analyses of metagenomes and metatranscriptomes, we have further shown that nanomolar N_2O consumption can occur in a sustainable manner in microoxic environments and also that the key players belong to the close relatives of the genera *Dechloromonas* and *Azospira*, the organisms that had been presumed to be high-affinity N_2O-reducing organisms. The presentation will provide an overview of these contributions of EML to the field in the past decade.

Session: Early Career Workshop 101
Presenter: Ayano Kaneko
Title: Isolation and physiological characterization of a novel nitrite-oxidizing bacterium, Nitrobacter sp. a67 which accommodates to moderately acidic pH from agricultural soil

Authors: Kaneko, Ayano; Endo, Yuta; Ninomiya, Takuya; Kuroiwa, Megumi; Fujitani, Hirotsugu; Suwa, Yuichi, Department of Biological Sciences, Chuo University, Tokyo, Japan

Nitrite oxidation is a key process in biogeochemical nitrogen cycle. Although resent studies suggest that nitrification occurs in acid soil, an adaptation to acid conditions of Nitrite-oxidizing bacteria (NOB) has not been fully understood. In this study, we report a novel isolate from agricultural soil affiliated with genus Nitrobacter and its physiological characteristics. We sampled surface soil of a vegetable field and obtained mixed culture A31 using the most probable number (MPN) method with inorganic medium containing 0.75 mM urea (pH 5.5). Microbial community analysis targeting the 16S rRNA gene in A31 revealed that ammonia-oxidizing archaea and NOB coexist. The dilution–extinction procedure from culture A31 led to the isolation of NOB, which was designated as Nitrobacter sp. A67. The nxrB1 fragment sequence of Nitrobacter sp. A67 (302 bp) was classified into Cluster 5, and the highest sequence identity was 96.10% with Nitrobacter sp. BS5/19. In physiology, changes in nitrite concentration by Nitrobacter sp. A67 at various pH (3.7, 4.2, 4.7, 5.3, 5.8, 6.4, 6.8, 7.3) was investigated. Media were buffered with phosphate – citrate buffer in adjustable pH 3.7 to 7.3. Growth of strain A67 was not observed below pH 4.7. High nitrite oxidation rates were measured at pH 5.3 and 5.8, while a lag time at pH 5.3 was prolonged. This result shows that strain A67 is a moderately acidophilic NOB. Currently, we plan to obtain biomass to analyze complete genome sequence of strain A67 and estimate mechanism of adaptation to acid conditions.

Session: Early Career Workshop 102
Presenter: Parker B. Lawrence
Title: Seasonal Dynamics of Marine and Estuarine Thaumarchaeota in Coastal North Carolina
Authors: Lawrence, Parker, B; Bolar, Bradley, B, Department of Biology and Marine Biology, University of North Carolina Wilmington

Estuaries are critical sites of biogeochemical cycling within river systems, particularly regarding nitrogen transformations. Nitrification, the two-step conversion of ammonia to nitrate via nitrite, is facilitated by a community of microbes from both Domain Archaea and Bacteria. Thaumarchaeota, a clade of non-extremophilic, ammonia-oxidizing archaea (AOA), contribute significantly to nitrification in virtually every biome on Earth. In Atlantic coastal systems along the southeastern USA, AOA are observed to annually bloom during the summer up to 3-4 orders of magnitude greater than their typical standing stocks. This sudden increase in ammonia oxidation outpaces nitrite oxidation and results in a decoupling of nitrification, leading to nitrite accumulation (≥10 μM). Here we investigate the temporal and spatial dynamics of such archaeal blooms in the Cape Fear River Estuary and along the North Carolina coast. Water from the estuary and an adjacent marine site has been collected monthly since March 2022, along with samples for nutrient analysis (dissolved inorganic nitrogen: ammonia, nitrite, and nitrate) and other environmental measurements (e.g., temperature, salinity, pH). From these samples, AOA abundance was measured with quantitative PCR (qPCR). Initial results revealed coinciding peaks of nitrite and archaeal gene copies at both sites—indicative of bloom dynamics. Future work seeks to determine the spatial extent of the bloom along the North Carolina coast and upstream of the estuary , as well as temporal dynamics at a finer scale across tides. Overall, our aim is to better understand environmental controls on microbially-mediated nitrogen cycling along a rapidly changing, human-impacted coastline.

Session: Early Career Workshop 103
Presenter: Marcel Malinowski
Title: Potential novel nitrifying bacteria from a freshwater recirculating aquaculture system (RAS) and their ecological niche formation

Authors: Malinowski, Marcel, Institute of Plant Science and Microbiology, Department of Microbiology & Biotechnology, Universität Hamburg, Hamburg, Germany; Spieck, Eva, Institute of Plant Science and Microbiology, Department of Microbiology & Biotechnology, Universität Hamburg, Hamburg, Germany

Recirculating aquaculture systems (RAS) offer a resource-saving and sustainable alternative to conventional fishing. Organic nitrogen accumulates by excess feeding and feces and toxic ammonium and nitrite are removed in biofilters, where nitrifying bacteria settle together with heterotrophic microorganisms on biocarriers and form dense biofilms. In most RAS, the key chemolithoautotrophic ammonia and nitrite oxidizing bacteria (AOB and NOB) are Nitrosomonas and Nitrospira. However, it has been shown that under certain circumstances Nitrotoga can also occur as a major NOB in aquaculture in coexistence with Nitrospira or even as the only nitrite oxidizer. The presence of Nitrosospira in RAS has already been shown, but it occurs rarely as the dominant AOB. In this study, the nitrifying consortium in an ammonia oxidizing culture from a freshwater aquaculture facility in Germany was analyzed. Enrichment was done over several years at low temperature (10°C), and was continued at 22 °C, after this was determined as the optimum temperature for the AOB. PCR-based analyses revealed the co-occurrence of Nitrosospira, Nitrospira and Nitrotoga. Based on the sequences of their functional genes (amoA and nxrB), Nitrosospira and Nitrospira are only distantly related to described species. Depending on the enrichment conditions with ammonium or nitrite as substrate, one of both NOB (Nitrospira and Nitrotoga) outcompeted the other one. Transmission and scanning electron micrographs confirmed this media-driven distribution of NOB, and the analysis of the metagenomes of the potential novel Nitrosospira and Nitrospira species are in progress to further analyze and uncover their phylogenetic affiliation and metabolic potential.

Session: Early Career Workshop 104
Presenter: Thomas Pribasnig
Title: The biofilm lifestyle of ammonia-oxidizing archaea: an understudied mode of growth and activity

Authors: Pribasnig, Thomas; Dreer, Maximilian, J; Hodgskiss, Logan, H; Kerou, Melina; Schleper, Christa, Department of Functional and Evolutionary Ecology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

Ammonia-oxidizing archaea (AOA) are ubiquitously distributed in soil and aquatic environments and through their autotrophic growth contribute significantly to both the global nitrogen and carbon cycles. Although, there is a continuously increasing number of axenic isolates available, comparably little is known about ecophysiological aspects and growth modes of AOA. In this study, Nitrososphaera viennensis was grown as a continuous culture in a bioreactor, where biofilm formation was observed under high flow rates. In order to better characterize growth in biofilm compared to planktonic growth, we performed a transcriptome study based on RNA-seq and were able to identify significantly distinct expression patterns between both growth modes, including (among others) genes for EPS production and adhesion being highly expressed in the biofilm. We then expanded our investigations to five strains of AOA representing different families (Nitrososphaeraceae, Nitrosocaldaceae, Nitrosopumilaceae) and ecosystems (marine, hot springs and terrestrial) to explore how widespread the ability to form biofilms is amongst the diversity of AOA. While all strains showed the ability, soil strains exhibited strongest biofilm formation. Our comparative analyses involving scanning electron microscopy (SEM) and confocal microscopy of biofilm grown on coverslips combined with expression studies of selected marker genes reveals distinct biofilm-forming abilities of AOA strains reflecting their phylogenetic distribution and ecological niches.

Session: Early Career Workshop 105

Presenter: Yuki Shiraishi

Title: Temperature dependence in nitrite oxidation and gene expression by a Nitrobacter sp.

Authors: Shiraishi, Yuki; Suwa, Yuichi; Fujitani, Hirotsugu, Department of Biological Sciences, Chuo University, Japan

Nitrite oxidizing bacteria (NOB) are important players in biotechnology. In nitrogen-rich agricultural fields and wastewater treatment plants, nitrite oxidation should properly be controlled to prevent nitrogen pollution and remove nitrogen efficiently. However, the slow growth rate of NOB at low temperatures could often cause insufficient nitrogen removal. Although it has fundamental importance, temperature dependence of NOB has not fully been examined. The aim of this study is to investigate the temperature dependence of a NOB isolate via the physiological characterization and transcriptome analysis. First, we investigated an activation energy to estimate the temperature dependence of nitrite-oxidizing Nitrobacter sp. strain CN101. The activation energy in the temperature range from 15 to 20℃ was calculated as 73.4 kJ/mol, and that in the from 20 to 30℃ was 14.5 kJ/mol. It is intriguing that the temperature dependence was considerably altered at about 20℃. Second, to understand a mechanism of the temperature dependence, we conducted a transcriptome analysis of strain CN101 grown at two different temperature conditions, 15℃ and 28℃. Consequently, many gene expressions were downregulated at 15℃. We found some functional genes were deeply relevant to an adaptability of strain CN101 to low temperature. This finding would provide us with biological understanding about temperature dependence of NOB.

Session: Early Career Workshop 106
Presenter: Alexander K. Umbach
Title: Succession of freshwater aquarium nitrifying communities in response to fish load
Authors: Umbach, Alexander, K; Szabolcs, Natasha; Sauder, Laura, A; Neufeld, Josh, D, University of Waterloo

Commercial and hobbyist aquarium systems depend on nitrifying microorganisms to reduce ammonia and nitrite concentrations, and newly established aquaria that lack established nitrifying communities are susceptible to toxicity-associated loss of aquatic life. Community succession for freshwater aquarium nitrifiers within biofilter microbial communities is poorly characterized. To investigate microbial community succession in response to fish load, aquaria containing varying numbers of zebrafish (i.e., Danio rerio) were established and maintained for 240 days. Ceramic beads from aquarium biofilters were collected throughout the experiment and extracted for genomic DNA. Whole-community 16S rRNA gene sequencing and nitrifier-targeted amoA qPCR analyses provided both general and specific microbial profiling of aquarium communities and abundances, respectively. Microbial community profiles from aquaria with high fish loads differentiated rapidly from low-fishload aquaria, within the first two weeks of aquarium operation, and remained distinct throughout the experiment. Differentiating taxa include members of the Planctomycetes, Flavobacterium, and Dadabacteriales. With respect to nitrifying communities, nearly all aquaria were consistently dominated by ammonia-oxidizing bacteria affiliated with Nitrosomonadaceae, regardless of fish abundance or aquarium operation time. Comammox Nitrospira were similarly present in most samples, although at relatively low abundance. In contrast to previous aquarium filter surveys, archaea were almost entirely absent from biofilter profiles, which may indicate that ammonia-oxidizing archaea (AOA) require introduction to establish a stable population within biofilter environments. Because our results contradict previous reports of dominant AOA and comammox populations within domestic freshwater aquarium biofilters, further investigation is needed to explore environmental influences on nitrifier immigration and establishment within freshwater aquaria.

Session: Biochemistry/Bioenergetics 201

Presenter: Justine Albers

Title: Adapting thermal shift assays to functionally characterize nitrifier substrate binding proteins

Authors: Albers, Justine, B., University of California, Santa Barbara; Santoro, Alyson, E., University of California, Santa Barbara

Transporter proteins enable cells to access essential compounds from dilute environments such as the oligotrophic ocean. Periplasmic substrate-binding proteins (SBPs) are components of ABC-transporters that are known for their high substrate specificity; however, in microbial genomes and metagenomes they are often annotated as binding broad classes of compounds. This limits our current understanding of cell physiology and oceanic nutrient cycling mediated by populations of uncultivated and genetically intractable microbes such as marine nitrifiers. To determine what kinds of compounds are being transported by these organisms, we are adapting thermal shift assays to functionally characterize SBPs in marine ammonia-oxidizing archaea (AOA) and nitrite-oxidizing bacteria (NOB). We identified SBPs with unverified substrate binding in the genomes of two cultivated marine AOA and three cultivated marine NOB and selected proteins annotated as “iron complex transport system” SBPs from Candidatus Nitrosopelagicus brevis and Nitrospira marina to perform initial assay validation. Using InterProScan, the two candidate protein sequences were further characterized as BtuF proteins, which are known to bind vitamin B12. Multiple sequence alignment of the nitrifier sequences with experimentally characterized E. coli BtuF verified that the Ca. N. brevis and N. marina SBPs contain residues for binding B12 and the corresponding ABC transporter subunit BtuC. E. coli BtuF was expressed and purified, and addition of 5 micromolar cyanocobalamin resulted in a thermostability increase of 7 degrees Celsius. Future work will optimize the purification and assay conditions of nitrifier BtuF proteins to verify that they bind B12.

Session: Biochemistry/Bioenergetics 202
Presenter: Jemma Fadum
Title: Using resource competition theory to explore the tradeoff between DNRA and denitrification in aquatic ecosystems
Authors: Fadum, Jemma M, Carnegie Science, Department of Global Ecology, Stanford, CA USA; Zakem, Emily J, Carnegie Science, Department of Global Ecology, Stanford, CA USA

Organic matter (OM) loading is a ubiquitous effect of net-pen aquaculture production and other anthropogenic perturbations to aquatic ecosystems, such as growing municipalities, intensifying industries, and expanding land use conversion. One important ecological impact of OM loading is that it alters the biogeochemical transformations that dictate the degree to which bioavailable N is retained in an ecosystem, rather than converted to nitrous oxide or dinitrogen gas. The dominance of a retentive pathway over a loss pathway for inorganic N can drive ecosystem scale changes, such as accelerated eutrophication. To improve our understanding and predictive abilities of these critically important transformations, we developed a microbial population model that mechanistically resolves the competition of dissimilatory nitrate reduction to ammonium (DNRA) and denitrification for nitrate (or nitrite). The redox chemistry underlying each metabolism informs the model parameters, providing a theoretically grounded quantitative framework. In the model, denitrification excludes DNRA when OM is limiting, but when the ratio of OM to nitrate supply is sufficiently high such that nitrate becomes limiting, DNRA can competitively exclude denitrification. This tradeoff is consistent with new observations from a lake with net-pen aquaculture production, previous chemostat experiments, and the empirically based understanding that DNRA rates dominate relative to denitrification in OM-rich environments. As the redox-constrained parameters may be more broadly applicable than species or location-specific parameters, our framework can aid in improving predictions of N cycling in environments where microbial community dynamics are largely unknown.

Session: Biochemistry/Bioenergetics 203
Presenter: Jessica Holechek
Title: In Vitro Assembly of Ammonia Monooxygenase into Nanodiscs
Authors: Holechek, Jessica, N., Cornell University Department of Chemistry and Chemical Biology; Laughlin, Alex, L., Cornell University Department of Chemistry and Chemical Biology; Lancaster, Kyle, M., Cornell University Department of Chemistry and Chemical Biology

Nitrification is mediated by ammonia-oxidizing bacteria (AOB) and archaea (AOA), which are vital to environmental nitrogen cycling. Despite the importance of this primary metabolism to global nitrogen balance, its constituent enzymatic steps remain largely unstudied. A key knowledge gap lies in the first step of nitrification, the selective oxidation of ammonia (NH3) to hydroxylamine (NH2OH) by ammonia monooxygenase (AMO). AMO initiates nitrification and is thus the primary gatekeeper of the global nitrogen cycle after N2 is fixed to NH3. AMO has long eluded chemical study due to the fact that AOB and AOA are slow-growing organisms that only grow to low cell densities. Thus, isolating active AMO from these organisms in biochemically useful quantities remains a challenge. Because of this, a recombinant expression system is desirable. However, no such platform has been described for the AMO complex due to the intrinsic challenges of expressing and purifying integral membrane proteins. As an alternative approach to study AMO, we have expressed and purified individual fusion constructs of each subunit of bacterial AMO (amoA, amoB, and amoC) in E. coli in high yield. We have reconstituted each of these subunits into lipid nanodiscs individually and are in the process of characterizing them via cryo-electron microscopy. Furthermore, we are working to assemble the full AMO complex through the combination of these individual subunits. Through this work, we aim to gain further insight on the initial step of nitrification and further elucidate the key enzymatic processes involved in environmental nitrogen cycling.

Session: Biochemistry/Bioenergetics 204
Presenter: Shuto Ikeda
Title: Growth heterogeneity of divided cells affects the culturability of ammonia-oxidizing bacteria

Authors: Shuto Ikeda, Department of Life Science and Medical Bioscience, Faculty of Advanced Science and Engineering, Waseda University; Hirotsugu Fujitani, Department of Biological Sciences, Faculty of Science and Engineering, Chuo University; Satoshi Tsuneda, Department of Life Science and Medical Bioscience, Faculty of Advanced Science and Engineering, Waseda University.

[Background] Most bacteria in environment are uncultured. In recent years, there have been reports of successful cultivation of uncultured bacteria by inducing re-division from a non-dividing state. Based on the above background, we hypothesized that the rate of transition to the nondividing state depends on strains, and strains that are difficult to culture may be more likely to transition to the non-dividing state. To prove this hypothesis, we observed i ndividual cell growth of two strains of ammonia-oxidizing bacteria with different culturability and compared the transition rates to the non-dividing state. [Methods] Nitrosomonas europaea NBRC 14298 (ATCC 19718) was used as the easily culturable AOB and Nitrosomonas sp. PY1 was used as the difficult-to-cultivate AOB. For observation, a microfluidic device was used in which cells were encapsulated and fixed between a membrane and a cover glass, and their growth could be observed at the individual level. [Results] In both strains, cell division was observed at the same generation time as when analyzed at the population level. On the other hand, non-dividing cells were also observed. In daughter cell pairs derived from the same parental cell, 9-24% of N. europaea pairs and 50-94% of Nitrosomonas sp. PY1 pairs had one daughter cell dividing while the other daughter cell transitioned to a non-dividing/slow-dividing state. These results suggest that difficult-to-culture strains of AOB (Nitrosomonas sp. PY1) are more prone to transition to the non-dividing state.

Session: Biochemistry/Bioenergetics 205
Presenter: Megumi Kuroiwa
Title: Quantitative evaluation of nitrous oxide sink capacity of denitrifying bacteria by a newly developed dual-tracer method

Authors: Kuroiwa Megumi, Department of Chemical Engineering, Tokyo University of Agriculture and Technology, Japan; Suwa Yuichi, Department of Biological Sciences, Chuo University, Japan; Oba Kohei, Department of Chemical Engineering, Tokyo University of Agriculture and Technology, Japan; Terada Akihiko, Department of Chemical Engineering, Tokyo University of Agriculture and Technology, Japan; Koba Keisuke, Center for Ecological Research, Kyoto University, Japan

Quantitative assessment of emissions and its pathways is needed to reduce N2O, a potent greenhouse gas. However, existing methods for analyzing fluxes and pathways of N2O emission are not quantitative and resolved enough to be applied to actual environments where multiple substrates coexist and N2O production and consumption occur simultaneously. In this study, we developed a new method to quantify N2O transformation rates and pathways using dual-labeled N2O with nitrogen and oxygen stable isotopes (15N, 18O) and 15N-labeled nitrogen compounds. We applied this method to two isolates of denitrifying bacteria: Alicycliphilus denitrificans strain I51 and Azospira sp. strain I13, to quantify the N2O emission and removal rate derived from each substrate under conditions of nitrite, nitrate, and N2O coexistence. In addition, the rate of dinitrogen production was also quantified to determine the percentage of the substrate utilized that was released outside the cell as N2O (Ce) for each substrate. In both strains, the emitted N2O was mainly derived from nitrite, and the Ce of nitrite was higher than that of nitrate by an order of magnitude or more. Al. denitrificans strain I51 always showed much higher N2O removal rate than N2O emission rate regardless of nitrite concentration, whereas Azospira sp. strain I13 only exerted as an N2O sink only at low nitrite concentrations and showed better N2O removal ability than Al. denitrificans under these conditions. The developed method would provide a new option to elucidate N2O dynamics in complex systems with N2O production from multiple substrates and/or multiple processes.

Session: Biochemistry/Bioenergetics 206
Presenter: Donald E. Martocello, III
Title: Physiological insights on bacterial and archaeal ammonia oxidation from variable stable isotope dynamics in response to metal limitation

Authors: Martocello, Donald, E.; Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA, United States & Department of Earth Atmosphere and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA; Wankel, Scott, D.; Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

The oxidation of ammonia to nitrite, the first and rate-limiting step of the globally important process of nitrification, is carried out by diverse ammonia oxidizing bacteria (AOB) and archaea (AOA). Both AOA and AOB require the metal micronutrients Cu and Fe for growth and enzymatic catalysis of ammonia oxidation. Presently, our knowledge of how metal availability limits or controls the physiological responses of AOB and AOA is limited – representing an important gap in our understanding of how nitrogen cycling may be regulated in many environments. Relatedly, while stable isotopes are widely used for constraining environmental cycling of nitrogen, it is unclear whether trace metal micronutrient availability may modulate expression of stable isotope fractionation during ammonia oxidation, whether in product nitrite or in production of trace N2O. Here we present the first study examining the influence of Fe and Cu availability on kinetic isotope effects in ammonia oxidation (15εAO) in both model AOB and AOA. We report an independence of 15εAO from growth rate as regulated by both Cu or Fe limitation in AOB Nitrosospira briensis C-128 and Nitrosococcus oceani C-107. In contrast, 15εAO values in AOA Nitrosopumilus maritimus (SCM1) exhibited notable response to Cu availability (but not Fe), with lower values under Cu-limitation. Our data suggest a tight regulation of core ammonia oxidation metabolic machinery, with variable sensitivity to Cu availability likely arising as the result of variations in mechanisms of NH4+ and NH3 uptake utilized by AOA and AOB.

Session: Biochemistry/Bioenergetics 207
Presenter: Benjamin Shapero
Title: Characterization of structural importance of nirk sequence diversity from AOA
Authors: Shapero, Benjamin, A., Stanford University; Francis, Christopher, A., Stanford University

Ammonia-oxidizing archaea (AOA) are key players in nutrient cycling, yet large gaps remain in our understanding of their biology. Multiple lines of evidence based upon (meta)transcriptomic, (meta)proteomic, biochemical, and physiological data suggest a central role for copper-containing nitrite reductase (NirK) in AOA metabolism. Consequently, this indicates that conservation of the structure/function of NirK, and therefore conservation of its amino acid sequence, should be essential for the fitness of these microorganisms. Yet, contrary to this expectation, nirK from AOA is not well conserved and instead exhibits large sequence diversity that correlates with the environmental distribution of these microbes. Here we use phylogenetics, structural prediction, and existing structures of NirK (from other organisms) to address this paradoxical relationship between the requirement for structural/functional conservation of NirK and its large sequence diversity. We find that approximately 20% of nirK sequence diversity occurs in the core structure of NirK (a duplicated cupredoxin fold) and is structurally degenerate leading to no change in the overall structure and therefore function of the enzyme. Meanwhile, approximately 80% of nirK sequence diversity occurs in intervening loops as well as N- and C-terminal extensions, leading to NirK structures with reduced active site accessibility. Interestingly, NirK structural predictions from soil and aquatic AOA suggest that these organisms achieve this same outcome via different structural modifications.

Session: Biochemistry/Bioenergetics 208
Presenter: Bongkeun Song
Title: Abiotic oxygen production from nitrite decomposition in acidic conditions
Authors: Song, Bongkeun, Virginia Institute of Marine Science; Cochran, Michele, Virginia Institute of Marine Science

The biological production of molecular oxygen is mainly mediated by photosynthetic organisms. Recently ammonia-oxidizing archaea were shown to produce trace amounts of oxygen while converting ammonia to nitrite. Nitrite-dependent anaerobic methane-oxidizing bacteria oxidize methane with molecular oxygen intercellularly produced by nitric oxide dismutation in the nitrite-reducing pathway. Chemodenitrification also utilizes nitrite abiotically converting to nitrous oxide and dinitrogen in acidic conditions. Chemical stoichiometry of chemodenitrification predicts oxygen production by nitrite self-decomposition. However, abiotic oxygen production has not been experimentally demonstrated. We conducted the incubation experiments of nitrite in anoxic phosphate buffers with different pH conditions ranging from pH 3 to 7. Oxygen production was monitored using the PreSens optical oxygen sensors while the changes in nitrite and nitrate concentrations were measured with ion chromatography. Trace amounts of oxygen were produced in the acidic solution but not in pH7 condition. Oxygen production immediately occurred within 4 hrs. and the concentrations of oxygen produced were inversely related to pH conditions. The highest levels of oxygen production were observed in the pH 3 solution. In addition, abiotic nitrate production was also detected in the acidic solutions. These results indicate that molecular oxygen production could occur much earlier than the appearance of photosynthetic organisms in early earth ecosystems.

Session: Biochemistry/Bioenergetics 209
Presenter: Hidetoshi Urakawa
Title: Nitrosomonas supralitoralis sp. Nov., a betaproteobacterial ammonia-oxidizing bacterium isolated from beach sand in a supralittoral zone, Washington, USA
Authors: Urakawa, Hidetoshi, Florida Gulf Coast University, Fort Myers, FL, USA; Andrews, Gabrianna, A., Florida Gulf Coast University, Fort Myers, FL, USA; Lopez, Jose, V., Nova Southeastern University, Dania Beach, FL, USA; Martens-Habbena, Willm, University of Florida, FL, USA; Klotz, Martin, G., Washington State University, Pullman, WA, USA; Stahl, David, A., University of Washington, Seattle, WA, USA

A betaproteobacterial chemolithotrophic ammonia-oxidizing bacterium designated APG5 was isolated from supralittoral sand of the Edmonds City Beach, Washington, USA. Growth was observed at 10-35°C (optimum, 30°C), pH 5-9 (optimum, pH 8), and ammonia concentrations as high as 100 mM (optimum, 1-30 mM NH_4Cl). The strain grows optimally in a freshwater medium but tolerates up to 400 mM NaCl. It is most closely related to Nitrosomonas ureae (96.7% 16S rRNA and 92.4% amoA sequence identity). The 3.75 Mbp of AGP5 draft genome contained a single rRNA operon, all necessary tRNA genes, a complete ure operon, and has the lowest G+C content (43.5 mol%) when compared to the previously reported genomes of reference strains in cluster 6 Nitrosomonas. Based on an average nucleotide identity of 82% with its closest relative (N. ureae Nm10T) and the suggested species boundary of 95~96%, a new species Nitrosomonas supralitoralis sp. nov. is proposed. The type strain of Nitrosomonas supralitoralis is APG5T (= NCIMB 14870T = ATCC TSD-116T).

Session: Biochemistry/Bioenergetics 210
Presenter: Bram Vekeman
Title: Versatile anaerobic ammonium-oxidizing bacteria can use alternative carbon and nitrogen sources for growth and energy conservation

Authors: Vekeman, Bram, Max Planck Institute for Marine Microbiology, 28359, Bremen, Germany; Kitzinger, Katharina, Max Planck Institute for Marine Microbiology, 28359, Bremen, Germany, Department of Microbiology and Ecosystem Science, University of Vienna, 1090 Vienna, Austria; Stührenberg, Jördis, Max Planck Institute for Marine Microbiology, 28359, Bremen, Germany; Marchant, Hannah, Max Planck Institute for Marine Microbiology, 28359, Bremen, Germany; Lavik, Gaute, Max Planck Institute for Marine Microbiology, 28359, Bremen, Germany; Kartal, Boran, Max Planck Institute for Marine Microbiology, 28359, Bremen, Germany, School of Science, Constructor University, Campus Ring 1, 28759, Bremen, Germany

Anaerobic ammonium-oxidizing (anammox) bacteria are chemolithoautotrophic microorganisms that use nitrite as the terminal electron acceptor to oxidize ammonium to dinitrogen gas. These microorganisms contribute substantially to the release of fixed nitrogen to the atmosphere, and are used for energy-efficient and environment-friendly nitrogen removal from wastewater. In both natural and engineered ecosystems anammox bacteria generally live under substrate limitation, and it was assumed that in nature anammox bacteria depend on other biochemical processes for its substrates. It has been shown that these microorganisms use small organic acids as an energy source, however it remains unclear whether they can directly grow on these substrates, and whether they are able to use alternative nitrogen species as substrates. In this study we enriched a new marine anammox bacteria (Scalindua sp.) from a sandy North Sea beach ecosystem, and tested its ability to conserve energy and grow using alternative carbon (formate, acetate, methanol) and nitrogen (cyanate, methylamine) species. We show that these anammox bacteria can use methylamine and methanol as electron donors, but the enzymes involved in their metabolism remain unknown. We further showed that this Scalindua sp. is able to directly assimilate formate and acetate for growth without the need to oxidize them to CO2 and reassimilation, suggesting that they are truly mixotrophic rather than being exclusively autotrophs. Together, these findings provide insight into the versatility of the carbon and energy metabolism of anammox bacteria and shed light into their survival strategies in nature.

Session: Biochemistry/Bioenergetics 211
Presenter: Rob Voland
Title: Structural and biochemical studies of novel enzymes implicated in nitrogen cycling
Authors: Voland, Rob, W, Cornell University

Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) survive by oxidizing ammonia to nitrite to produce energy, however, there are many enzymological "black boxes" in these systems. While much is known about the metabolism of canonical AOB and the operative metalloenzymes involved, very little is known about the pathway and enzymes involved in the respective metabolisms of comammox bacteria, which can convert ammonia to dinitrogen, or AOA. It is well known that hydroxylamine oxidoreductase (HAO) in AOB uses a c-type heme in a cytochrome P460 cofactor to oxidize hydroxylamine to nitric oxide (NO). The same is not true for AOA, as AOA lack c-type heme maturation machinery. Here, we show our structural and biochemical approaches to elucidate and study the enzymes involved in both commamox and AOA metabolic pathways. We have expressed, purified, and studied an enzyme thought to be central to comammox metabolism and successfully expressed and purified many AOA enzymes which have been implicated in hydroxylamine and NO processing via genomic analysis. We have identified a multi-copper oxidase from the marine archaeon Nitrosopumilus maritimus with interesting reactivity toward phenolic amines and hydroxylamine and a seemingly novel structure containing a unique C-terminal cupredoxin domain. Understanding the operative enzymes in these systems on a structural and biochemical basis can help us better understand how these organisms perform such difficult, yet essential, chemistry and can help us better understand the evolution of these impressive organisms.

Session: Biochemistry/Bioenergetics 212
Presenter: Wei Qin and Britt Abrahamson
Title: Ammonia-oxidizing microorganisms employ distinct strategies coordinating ammonia and urea metabolism

Authors: Wei Qin[1, 2†*], Stephany P. Wei[2†], Yue Zheng[3†], Eunkyung Choi[4†], Xiangpeng Li[1], Juliet Johnston[5], Xianhui Wan[6], Britt Abrahamson[2], Zachary Flinkstrom[2], Baozhan Wang[7], Hanyan Li[1], Lei Hou[1,3], Qing Tao[1], Michael Chlouber[1], Xin Sun[8], Michael Wells[1], Long Ngo[1], Kristopher A. Hunt[2], Hidetoshi Urakawa[9], Xuanyu Tao[1], Dongyu Wang[1], Dazhi Wang[3], Chongle Pan[1], Peter K. Weber[5], Jiandong Jiang[7], Jizhong Zhou[1], Yao Zhang[3], David A. Stahl[2], Bess B. Ward[6], Xavier Mayali[5], Willm Martens-Habbena[4*], Mari -Karolina H. Winkler[2*]

[1]Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, OK, USA
[2]Department of Civil and Environmental Engineering, University of Washington, Seattle, WA, USA.
[3]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian, China.
[4]Department of Microbiology and Cell Science, University of Florida, Davie, FL, USA.
[5]Lawrence Livermore National Laboratory, Livermore, CA, USA.
[6]Department of Geosciences, Princeton University, Princeton, NJ, USA.
[7]College of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu, China.
[8]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA.
[9]Department of Ecology and Environmental Studies, Florida Gulf Coast University, Fort Myers, FL, USA.
[†]These authors contributed equally to this work: Wei Qin, Stephany P. Wei, Yue Zheng, Eunkyung Choi

Ammonia oxidation, the first step pf nitrification, is a low energy-yielding process now known to be carried out by four phylogenetically distinct lineages of autotrophic Archaea and Bacteria with varying affinities for ammonia. Although different lineages of ammonia-oxidizing microorganisms (AOM) are thought to compete for their primary N substrate, ammonia, they often grow concomitantly in various habitats, raising the question of other adaptive strategies that allow for their coexistence. Urea is an important and widely available organic nitrogen compound in aquatic and soil environments, and many AOM species can use urea as an alternative substrate, however, it is unknown how nitrogen source selection is regulated in AOM species. Using a combination of stable isotope tracing, nanoSIMS, genome-wide gene expression, and kinetic measurements we show that the characterized ammonia-oxidizing archaea (AOA) and complete ammonia oxidizer (comammox) species preferentially used ammonia over urea. In contrast, the characterized beta-proteobacterial ammonia-oxidizing bacteria (AOB) used urea as preferred energy and nitrogen source, and gamma-proteobacterial AOB utilize ammonia and urea simultaneously. Stable isotope tracking confirmed that both ammonia oxidation and assimilation are transport-dependent, suggesting a cytoplasmic entry site of the ammonia monooxygenase in AOM. Together, our study reveals an unexpected diversity in substrate preference and supporting regulatory systems among AOM and provides new understanding of division of labor among AOM that drive nitrification in present-day natural and engineered ecosystems.

Session: Biotechnology/Applications 301
Presenter: Eleftheria Bachtsevani
Title: In vitro assessment of pesticide toxicity on soil nitrifying microorganisms: a novel tool for ecotoxicity risk assessment
Authors: Eleftheria Bachtsevani, Environmental Microbial Genomics Group, Laboratoire Ampère, École Centrale de Lyon, CNRS UMR 5005, Université de Lyon, Ecully, France; Maria Kolovou, Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece and Laboratory of Biotechnology and Environmental Microbiology, Department of Environmental Sciences, University of Thessaly, Larissa, Greece; Evangelia Papadopoulou S., Laboratory of Biotechnology and Environmental Microbiology, Department of Environmental Sciences, University of Thessaly, Larissa, Greece; Dimitrios Karpouzas G., Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece; Christina Hazard, Environmental Microbial Genomics Group, Laboratoire Ampère, École Centrale de Lyon, CNRS UMR 5005, Université de Lyon, Ecully, France; Graeme W. Nicol, Environmental Microbial Genomics Group, Laboratoire Ampère, École Centrale de Lyon, CNRS UMR 5005, Université de Lyon, Ecully, France

Pesticides are considered major environmental pollutants and their toxicity is determined with toxicity assays for aquatic and terrestrial organisms. However, there are no corresponding bioassays for soil microorganisms due to the lack of identified microbial indicators, and standardized in vitro tests which could be used as standard tier I type assays equivalent to single species tests used in aquatic ecotoxicology. Recent studies have indicated that ammonia-oxidizing microbes are ideal microbial indicators of the effects of agrochemicals on soil microbial communities due to their key functional role, their sensitivity to xenobiotic compounds, and the availability of established tools to measure their activity and abundance. We aimed to characterize the sensitivity of soil nitrifiers to pesticide compounds and evaluate their use as bioindicators using in vitro assays for a tier I approach in pesticide risk assessment. Relative toxicity thresholds (EC50s) were determined via monitoring nitrite production or consumption in liquid cultures by ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and nitrite-oxidizing bacteria, respectively, representing globally distributed lineages found in soil. All tested pesticides affected at least one nitrifier, with the different strains exhibiting various sensitivity levels. For AOB, pesticides were most toxic for Nitrosomonas ureae and Nitrosospira briensis, while Ca. Nitrosotalea sinensis was the most sensitive AOA strain. NOB strains were less affected than their ammonia-oxidizing counterparts, with Nitrobacter sp. NHB1 being the most sensitive. Our findings are expected to benchmark the development of novel ecotoxicity tools and a risk assessment scheme characterizing the impacts of pesticide on non-target soil microbes.

Session: Biotechnology/Applications 302
Presenter: Jiyun Li
Title: Comammox Nitrospira dominated in a low dissolved oxygen nitrifying reactor
Authors: Zilu Ouyang, Jiyun Li, Tingting Zhang, Chengwen Wang, Tsinghua University

The biological nitrification process is high energy consumption step in wastewater treatment plant (WWTP). This process is completed in two steps by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) with dissolved oxygen （DO) above 2.0 mg/L. The complete ammonia oxidizing (comammox) was discovered to catalyse ammonium to nitrate alone. The isolated pure culture Nitrospira inopinata showed that it can adapt to growth in oligotrophic and dynamic conditions. In this study, a continuous membrane bioreactor was operated under low DO conditions to evaluate the nitrification performance and identify nitrifying populations. The influent ammonium concentration was around 50 mg/L and organics was not added into the influent. The hydraulic retention time was 12 h and there was no sludge discharged except for the sludge samples. DO in the reactor was controlled by an automatic control system, thus daily average DO was 0.1-0.3 mg/L. The effluent ammonium removal rate reached to nearly 100%. Nitrite concentrations were almost negligible and nitrate concentrations were around 50 mg/L. These results showed that the reactor had high ammonium removal efficient under low DO conditions. Utilizing 16S rRNA gene amplicon sequencing, the relative abundance of Nitrospira was 64.86% and dominated in the low DO reactor, far more than AOB (0.06%). These results indicated that comammox Nitrospira likely play a crucial part in the low DO reactor. Thus low DO nitrification operation seems to be an energy saving strategy in WWTP.

Session: Biotechnology/Applications 303
Presenter: Mohammad H. Mozaffari
Title: Influence of media size and plant species on nitrification in unsaturated vertical flow wetlands
Authors: Mozaffari, Mohammad H.; Allen, Christopher R.; Lauchnor, Ellen G.; Stein, Otto R., Department of Civil Engineering and Center for Biofilm Engineering, Montana State University, Bozeman, MT, 59717 USA

The unsaturated second stage of a two-stage vertical flow (VF) treatment wetland is commonly used to enhance nitrification of domestic wastewater after most organic carbon (BOD, COD) is removed in the first stage. A pilot system in operation since 2013 has shown nearly complete nitrification at temperatures as low as 2°C. To better characterize effects of media size and plant presence on nitrification efficiency, 24 vertical flow mesocosms (diameter=20 cm, height= 50 cm) are subjected to an annual temperature cycle between 0.5˚C and 24˚C. Mesocosms contain either washed sand (d50 ~ 0.4 mm) or crushed gravel (d50 ~ 5 mm) as media and planted with Phragmites australis, or Carex utriculata or left unplanted (4 replicates each combination). Mesocosms are dosed with 500 ml of an ammonium-rich (109-115 mg/l NH_4^+-N) solution devoid of organic carbon 12 times daily, idealizing flow to a second stage VF (HLR= 0.19 m/d; N load rate= 21 g N/m^2·d). Initial results at 4°C and 8°C indicate that the sand mesocosms discharged 42-46% and 46-52% of the incoming ammonium as nitrate respectively, while the gravel columns converted only 13-18% and 17-25% of the influent ammonium, respectively. The presence of plants increased the observed nitrification efficiency only slightly within a specific media. Media differences are likely due to the differences in specific retention of water while differences between plant species are expected to increase as plants mature. Data collected until the time of the conference will be presented and compared to long-term results of the pilot.

Session: Biotechnology/Applications 304
Presenter: Satoshi Okabe
Title: Oxygen tolerance and detoxification mechanisms of anammox bacteria
Authors: Satoshi Okabe[1*], Shaoyu Ye[1], Lan Xi[1], Keishi Nukada[1], Haozhe Zhang[1], Kanae Kobayashi[12], and Mamoru Oshiki[2]

1 Department of Environmental Engineering, Faculty of Engineering,
Hokkaido University. North-13, West-8, Kita-ku, Sapporo Hokkaido, 060-8628, Japan
2 Super-cutting-edge Grand and Advanced Research (SUGAR) Program, Japan Agency for
Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka city,
Kanagawa, 237-0061, Japan

Oxygen is a key regulatory factor of anaerobic ammonium oxidation (anammox). We determined the oxygen tolerance and detoxification mechanisms of four genera of anammox bacteria; one marine species (“Ca. Scalindua sp.”) and four freshwater anammox species (“Ca. Brocadia sinica”, “Ca. Brocadia sapporoensis”, “Ca. Jettenia caeni”, and “Ca. Kuenenia stuttgartiensis”) and then related to the activities of anti-oxidative enzymes. A marine species, “Ca. Scalindua sp.”, exhibited the higher aerotolerance and reversibility than other freshwater anammox species. This is primarily because “Ca. Scalindua sp.” possesses the classical Sod-Cat ROS detoxification system. The upper DO limit for “Ca. Scalindua sp.” was much higher than the values reported so far. This might suggest that the contribution of anammox to oceanic nitrogen loss could be larger than we have ever thought.

Session: Biotechnology/Applications 305

Presenter: Yung-Hsien Shao

Title: Comammox Nitrospira Cooperate with Anammox Bacteria in a Partial Nitritation–Anammox Bioreactor Treating Low-strength Ammonium Wastewater at High Loadings

Authors: Yung-Hsien Shao. Department of Environmental Engineering, National Cheng Kung University, Taiwan; Huei-Wen Chen. Department of Environmental Engineering, National Cheng Kung University, Taiwan; Jer-Horng Wu*. Department of Environmental Engineering, National Cheng Kung University, Taiwan

Recent studies have revealed the co-occurrence and potential cooperation of comammox Nitrospria and anammox bacteria in partial nitritation-anammox (PN-A) systems. However, this cooperative partnership and the corresponding performance of treating low-strength ammonium wastewater are yet to be determined. In this study, a membrane bioreactor fed with synthetic ammonium wastewater (60 mg-N/L) was operated under hydraulic retention time (HRT) stepwise shortened from 79.6 to 4 hours, reaching the highest loading of 0.36 kg-N/m3/day with 78±4.8% of nitrogen removal efficiency under specific intermittent aeration conditions. The stoichiometric analysis of nitrogen conversion revealed successful PN-A and suppression of nitrate accumulation. Quantitative polymerase chain reaction analyses suggested that comammox Nitrospira dominated at low loadings while outnumbered by ammonia-oxidizing bacteria (AOB) at high loadings (HRT≤12 hours). However, the results of the batch assay with selective inhibitors, 1-octyne to inhibit AOB and chlorate to inhibit Nitrospira, showed that comammox Nitrospira, despite a lower abundance, performed a specific ammonia-oxidizing rate (18.3±1.1 mg-N/g-VSS/hr) higher than that of AOB (12.6±1.8 mg-N/g-VSS/hr) and substantially surpassing the nitrite-oxidizing rate (3.0±0.3 mg-N/g-VSS/hr). When dosed with 1-octyne to inhibit AOB activities in the reactor, 54.8±9.5% of the input ammonium was removed from the reactor, and only 11.0±4.1% of removed ammonium was oxidized to nitrate under HRT of 4 hours. The inhibitor test results validated the cooperation of comammox and anammox bacteria for converting ammonium to nitrogen gas. This study experimentally demonstrated using a PN-A membrane reactor to effectively treat low-strength ammonium wastewater at high loadings under the cooperation of comammox and anammox bacteria.

Session: Biotechnology/Applications 306
Presenter: Christiana Abiola
Title: Differential growth of ammonia-oxidizing archaea on floating filter

Authors: Abiola Christiana; Gwak Joo-Han; Lee Ui-JU; Adebisi Olabisi; Si Ok-Ja; Rhee Sung-Keun*; Department of Biological Sciences and Biotechnology, Chungbuk National University, 1 Chungdae-ro, Seowon-Gu, Cheongju 28644, Republic of Korea

Ammonia-oxidizing Archaea (AOA) belonging to the class Nitrososphaeria, affiliated to the phylum Thermoproteota formerly known as Thaumarchaeota are ubiquitous and abundant in the terrestrial, marine, and geothermal ecosystems. So far, there is no report on the growth of AOA on floating filters. The aim of this study was to develop novel strategies for the cultivation of AOA from terrestrial environments. We tested the growth of two phylogenetically distinct AOA strains: Nitrososphaera viennensis EN76 and "Candidatus Nitrosotenuis chungbukensis˝ MY2 on a floating filter. At the same time, it was compared with those in liquid medium. Growth of N. viennensis EN76 on a polycarbonate filter was observed as ammonia was stoichiometrically converted to nitrite in Artificial Freshwater Medium (AFM) below the filter, colonies were not visible with the naked eye. In contrast, "Ca. N. chungbukensis˝ MY2 growth was not observed on the floating filter. After the growth of strain EN76, the morphology of N. viennensis EN76 microcolonies on the filter was ascertained via fluorescence microscopy. We look further to investigate possible gene expression in N. viennensis EN76 grown on the floating filter as compared with those in liquid AFM by using transcriptomic analysis. These findings shed new light on the growth of AOA on solid soil particles as well as expand our knowledge on developing new cultivation techniques for novel clades of AOA from terrestrial environments.
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Session: Biotechnology/Applications 307
Presenter: Han-Yin Chuang
Title: Effects of glutamate on population dynamics of anammox consortia associated with salinity changes

Authors: Han-Yin Chuang, Muhammad Naufal, Yung-Hsien Shao, and Jer-Horng Wu*
Department of Environmental Engineering, National Cheng Kung University, Taiwan.
*Corresponding author (enewujh@ncku.edu.tw)

High salinity can substantially deteriorate the activity of freshwater anammox bacteria. A recent genomic and experimental study has revealed that the osmotic stress of anammox bacteria can be alleviated by enhancing the osmoadaptation capability with the addition of glutamate. However, adding glutamate to the anammox consortia can lead to the competition of heterotrophic bacteria. This ecological effect corresponding to the pristine sludge remains unknown. In this study, two sequencing batch anammox reactors were operated with sodium glutamate (0.2-1mM), one with freshwater conditions and the other with gradually increasing salinity. After 146 days of operation, the freshwater reactor achieved a nitrogen removal of 88.2±3.5% with Ca. Kuenenia abundance increased from 27.3% to 55.7%. The saline reactor maintained high anammox-based nitrogen removal at low salinity (0.5-1.5%), but the Ca. Kuenenia activity started to decline at 2.5% salinity and ultimately collapsed at 3.5%. The Ca. Kuenenia abundance markedly decreased by 37%, while the consortium was subsequently dominated by the halophilic Halomonas for denitrification-based nitrogen removals with nitrite. The transcripts of the hzsA and nirK genes showed a similar trend, with a higher gene expression of the nirK than the hzsA at 3.5% salinity. These results indicated that Ca. Kuenenia was more susceptible to rising salt levels than heterotrophic bacteria, even with glutamate. The developed anammox activity of pristine sludge in salinity <2.5% was not hindered by the glutamate addition. Overall, this study expands the understanding of the osmoadaptation of anammox consortia and facilitates the application of anammox processes in treating ammonium-rich saline wastewater.

Session: Biotechnology/Applications 308

Presenter: Akito Oishi

Title: Genomic and physiological insights into N2O emissions by cluster 7 ammonia-oxidizing bacteria isolated from partial nitrifying bioreactors

Authors: Akito Oishi, Tokyo Univ. Agr. & Technol., Satoru Ono, Tokyo Univ. Agr. & Technol., Toshikazu Suenaga, Tokyo Univ. Agr. & Technol., Hirotsugu Fujitani, Chuo Univ., Yuichi Suwa, Chuo Univ., Megumi Kuroiwa, Tokyo Univ. Agr. & Technol., Akihiko Terada, Tokyo Univ. Agr. Technol.

Cluster 7 ammonia-oxidizing bacteria (AOB) are often detected in wastewater treatment bioreactors receiving high nitrogen concentrations. While these AOB possess a high ammonia conversion capacity, nitrous oxide (N2O) production rates are generally high. Despite its significance in high-rate nitrification, N2O emissions and their mechanisms have not been thoroughly investigated. Therefore, this study was undertaken to evaluate the genomic and physiological characterizations of two cluster 7 AOB isolated from partial nitrifying reactors continuously (CSTR) and intermittently (SBR) fed with 500 mg-N/L of ammonia with an identical loading rate. Hybrid genome assembly and annotation revealed that CSTR and SBR strains are 98.22% and 98.63% identities to Nitrosomonas europaea ATCC19718 and Nitrosomonas sp. HPC101, respectively. Both strains possess the same functional genes responsible for nitrogen conversions except nitric oxide (NO) reduction: CSTR and SBR strains harbor cnor and qnor, respectively. The microrespirometory analysis for O2, NO, and N2O production dynamics showed that N2O production in both strains was intensified after oxygen depletion. SBR strain sharply increased and decreased NO concentration under an anoxic condition, whereas CSTR strain did not. N2O production after oxygen depletion by CSTR strain was more pronounced, with the highest N2O concentration 14 times higher than in that by SBR strain. The results indicate that N2O emissions differ, likely depending on the two types of nor. A deep understanding of the ecophysiology of cluster 7 AOB may allow harnessing AOB to produce less N2O, contributing to the mitigation of N2O emissions without compromising high-performance nitrogen removal.

Session: Biotechnology/Applications 309
Presenter: Mamoru Oshiki
Title: Remineralization of organic nitrogen compounds into ammonium in marine anammox bacterial culture
Authors: Oshiki, Mamoru, Hokkaido University; Morimoto, Emi, Hokkaido University; Kobayashi, Kanae, Hokkaido University, X-star, JAMSTEC; Satoh, Hisashi, Hokkaido University; Okabe, Satosh, Hokkaido University

The global nitrogen cycle is a pivotal biogeochemical cycle on the Earth, and massive nitrogen loss is occurring in the ocean. Oxygen minimum zones (OMZs) have been recognized as the hotspots of the oceanic nitrogen loss, because 25-35% of the oceanic nitrogen loss occurred in the OMZs although the OMZs accounts for <1% of the ocean volume (when defined by O2 £20 µM). Anammox process greatly contributes to the nitrogen loss occurring in OMZs, whereas the availability of ammonium is generally scarce in the OMZ core as compared with nitrite. Remineralization (i.e., degradation) of organic nitrogen compounds including urea and cyanate into ammonium has been suggested as a primal ammonium source of the anammox process in OMZs, while occurrence and the activities of urea- or cyanate-dependent anammox have not been well characterized and are still controversial due to the lack of marine anammox bacterial culture. We have successfully enriched a marine anammox bacterium, Candidatus Scalindua sp., using a membrane bioreactor, and their abundance was further increased >99.9% of the total cells by buoyant density centrifugation. This highly-enriched Scalindua sp. biomass provided an excellent opportunity to examine the urea and OCN- metabolism of marine anammox bacteria. In the present study, urea and cyanate degradation by marine anammox bacterium, Candidatus Scalindua sp., were investigated by 15N-tracer techniques and the metabolic potentials of urea and cyanate degradation were further examined by metagenomic analysis.

Session: Biotechnology/Applications 310

Presenter: Lisa Stein

Title: Inoculum and pH effects on ammonium removal and microbial community dynamics in aquaponics systems

Authors: Derikvand, Peyman, Dept. Biological Sciences, Univ. Alberta, Edmonton AB Canada; Sauter, Brittany, Dept. Biological Sciences, Univ. Alberta, Edmonton AB Canada; Keddie, Andrew, Dept. Biological Sciences, Univ. Alberta, Edmonton AB Canada; Stein, Lisa Y., Dept. Biological Sciences, Univ. Alberta, Edmonton AB Canada

Understanding the ecology of microorganisms is essential for optimizing aquaponics systems. Effects of pH and inoculum on ammonium removal and dynamics of microbial community composition from all compartments of lab-scale aquaponics systems were examined. Initial ammonium accumulation in systems with comammox-enriched inocula were 47 and 69% that of systems enriched with ammonia-oxidizing bacteria (AOB), with higher rates of ammonium removal and transient nitrite accumulation measured in the latter systems. By the end of operation, Nitrosomonas and Nitrosospira AOB were dominant nitrifiers in systems at pH 7.6-7.8, whereas comammox (Nitrospira) nitrifiers and plant growth-promoting microbes were abundant in systems operating at pH 5.8-6.0. Lower pH systems supported more robust plant growth with no significant effects on fish health. This study demonstrated functional redundancy of aquaponics microbiota, with selectivity of nitrifying taxa as a function of pH. Results suggest that inoculum and pH are important considerations for aquaponics system initiation and optimization.

Session: Biotechnology/Applications 311
Presenter: David Vuono
Title: Continuous Measurements of NOB Growth through the application of photoconduction in a Microbial Growth Intervalometer (MAGI).
Authors: Vuono, David, C, Colorado School of Mines; Flinkstrom, Zach, University of Washington; Abrahamson, Britt, University of Washington; Staub, Carl, Lumenautix LLC; Henriikka Winkler, Mari-Karoliina, University of Washington

A widespread problem with traditional optical density measurements is the inability to detect cultured organisms with low cell yields, or optically translucent and small cells, often a feature of organisms found in the nitrogen cycle. This problem often requires investigators to use time consuming methods to ascertain cell counts, growth curves, and growth rates. To solve this problem, a high-sensitivity optical density device was invented. This device, called a Microbial Growth Intervalometer (MAGI), uses photoconduction of actively growing cells and a resulting signal that is proportional to cell yield, in traditional Balch-type tubes. The ability of a MAGI to quantify the growth of nitrifying organisms was evaluated with several nitrite oxidizing bacteria (NOB) isolates. Growth of the NOB isolates on 1mM nitrite were either monitored continuously measured with a MAGI or periodically with a cytometer or microscopic cell counting. Differences between the NOB growth rates measured with a MAGI or traditional cell counting methods were found to be statistically insignificant. These results indicate the MAGI can accurately quantify growth rates after an organism specific calibration procedure.

Session: Biotechnology/Applications 312
Presenter: Shih-Wei Yeh
Title: Membrane oxygenation-driven anammox biofilm reactor treating low-strength ammonium wastewater

Authors: Yeh, Shih-Wei, Department of Environmental Engineering, National Cheng Kung University, Taiwan; Shao, Yung-Hsien, Department of Environmental Engineering, National Cheng Kung University, Taiwan; Wu, Jer-Horng, Department of Environmental Engineering, National Cheng Kung University, Taiwan

Partial nitritation/anammox is a sustainable biotechnology to treat ammonium wastewater. However, because of imprecise bubble aeration to supply oxygen for partial nitritation, its application to treat low-strength ammonium wastewater is challenging. This study established a membrane oxygenation biofilm reactor to perform the partial nitritation/anammox process for treating low-strength ammonium wastewater. The breathable membrane supplies oxygen from the dry side with low energy consumption but high oxygen transfer efficiency to the biofilm attached to the wet side of the membrane. After optimizing oxygenation parameters, including duration, frequency, and flowrate of air supply, the reactor achieved an efficient nitrogen removals of >70% when treating synthetic wastewater with input ammonium-nitrogen of 80 mg/L. The aerobic ammonium oxidation rate of 3.82 mgN/gSS/hr was substantially higher than the aerobic nitrite oxidation rate of 0.1 mgN/gSS/hr, suggesting a successful partial nitritation of ammonium through precise membrane oxygenation. Redundancy analysis revealed that the anammox rate was positively correlated with aeration frequency but negatively correlated with aeration duration. The reactor was then tested with industrial wastewater at a loading of 80 mgNH4+-N/L/day, achieving high COD and nitrogen removals with 50% and 75% efficiencies, on average, respectively. Sequencing of 16S rRNA gene amplicons showed that relative abundances of nitrifying and anammox bacteria in the membrane-attached biofilms were 1.2 and 3.4 times higher than suspended sludge and 1.3 and 3.2 times higher than wall-attached biofilms, respectively. These results demonstrated that the membrane oxygenation biofilm reactor is a promising model for implementing a partial nitritation/anammox process to treat low-strength ammonium wastewater.

Session: Biotechnology/Applications 313
Presenter: Mingsheng Jia
Title: Does AOA-driven thermophilic nitritation have lower N2O emissions?

Authors: Jia Mingsheng[1,2], Zeng Lin[1,2], Ravikumar Badri Narayan[1,2], Vlaeminck Siegfried[2,3], Volcke Eveline[2,4], Carvajal Arroyo Jose Maria[1,5], Boon Nico[1,2], Ganigué Ramon[1,2]

1 Centre for Microbial Ecology and Technology (CMET), Ghent University, Belgium
2 Centre for Advanced Process Technology for Urban Resource Recovery (CAPTURE), Belgium
3 Department of Bioscience Engineering, University of Antwerp, Belgium
4 BioCo Research Group, Department of Green Chemistry and Technology, Ghent University,
5 Colsen B.V., Hulst, The Netherlands

Nitrification plays a crucial role in global nitrogen cycle and biological nitrogen removal from wastewater. The first step, ammonia oxidation to nitrite (nitritation), is conducted by ammonia-oxidizing bacteria (AOB) or archaea (AOA), or complete ammonia oxidizers. While most AOB thrive under mesophilic conditions, thermophilic nitritation is mainly carried out by AOA. Physiological characteristics, including mechanisms for nitrous oxide (N2O) production, differ within and between AOA and AOB. Since AOA typically have lower N2O yields, thermophilic nitritation may emit less N2O than its AOB-driven counterpart. Nevertheless, data on this aspect is currently lacking. Furthermore, recent enrichment of a thermophilic anammox culture opens the door to develop innovative resource-efficient thermophilic partial nitritation-anammox process for directly treating nitrogen-rich warm wastewaters. Although strategies have been developed for mesophilic nitritation, how to achieve AOA-driven thermophilic nitritation remains unexplored. This contribution provides a proof of concept of thermophilic nitritation process and delivers quantitative data on its N2O emissions under different conditions. Using a membrane bioreactor, a mixed culture thermophilic nitritation process was established from full nitrification at 50°C for the first time, induced by a low pH (5.7) shock. The dominant AOA and nitrite-oxidizing bacteria belonged to the genera Candidatus Nitorsosphaera and Nitrospira, respectively. Data from comprehensive online monitoring of N2O in both gas and liquid phases suggested lower N2O emissions (0.6-1.5% g N2O-N/g NH4-N converted) than typical mesophilic nitritation. This study lays the groundwork for the development of sustainable thermophilic nitrogen removal processes and enables more holistic evaluations of their carbon footprint.

Session: Terrestrial/Soil/Agriculture 401
Presenter: Shannon Brown
Title: Linkage of nitrification and denitrification and its impact on carbon cycling in tropical agricultural soils
Authors: Brown, Shannon, University of Florida, Zhao, Jun, University of Florida; Infantado, Nina, University of Florida; Daroub, Samira, University of Florida; Martens-Habbena, Willm, University of Florida

Tropical agricultural soils are vulnerable to nutrient loss and often become unfertile within few decades after initial cultivation, requiring large inputs of fertilizer for continued productivity. Tropical soils also are prone to loss of soil carbon due to excess respiration. We aimed to quantify the role of nitrification and denitrification in N loss in two contrasting tropical agricultural soil in South Florida relative to agricultural management. We used a highly fertile organic histosols soil in the Everglades Agricultural Area (EAA) in Belle Glade, FL and a highly fertilized, sandy soil in Clewiston, FL. Metatranscriptomic analyses indicated substantial expression of denitrification pathway genes even in soils devoid of NO3-. Potential and net nitrification rates, denitrification rates, and carbon mineralization rates were determined in microcosm incubations. Carbon mineralization rates ranged between 0.15 to 3.64, and 0.18 to 0.63 µmol C / g dw soil / day in EAA soils and Clewiston soils, respectively. Nitrification rates correlated with soil NO3- concentrations, and net denitrification rates were below 6.3 x 10-4 µmol N / g dw soil / day, but could be stimulated under anoxic conditions in nitrate-containing soils and by addition of 100µg KNO3-N / g dw soil. Carbon mineralization rates were reduced by 20-50% under strictly anoxic conditions, compared to oxic incubations. Expression of denitrification pathway genes remains high following NO3- depletion. Together, the results suggest that succession of dry season nitrification and wet season denitrification can be a significant N sink. Denitrification gene expression remains surprisingly stable long after NO3- depletion.

Session: Terrestrial/Soil/Agriculture 402
Presenter: Hirotsugu Fujitani
Title: Physiology and genomics of a soil nitrite-oxidizing bacterium, Nitrobacter sp. Cn101 with high affinity to nitrite

Authors: Fujitani, Hirotsugu; Kobayashi, Yoichiro; Ninomiya, Takuya; Kuroiwa, Megumi; Suwa, Yuichi; Department of Biological Sciences, Chuo University, Japan

Nitrite is a key intermediate in global nitrogen cycles, but its accumulation is often not appreciable in environment, even though nitrite oxidation could be active. However, nitrite concentrations in media for cultivating nitrite oxidizing bacteria (NOB) in laboratories may not be low enough to recover oligotrophic NOB. In this study, we newly designed a medium containing urea as a sole energy source for enriching oligotrophic NOB. NOB in a Japanese forest soil sample were enumerated by most probable number method. It is notable that urea was completely converted into nitrate and no nitrite was detected in all the nitrifier-positive-tubes cultivated after 9 weeks of incubation. After subculturing of several times in mineral medium supplemented with 1 mM nitrite and extinction-dilution procedure, a novel strain oxidizing nitrite to nitrate was obtained and designated as strain CN101, which was affiliated with genus Nitrobacter in 16S rRNA gene level. The half saturation constant, Km value was orders of magnitude lower than previously reported values for other known Nitrobacter strains, and in the similar level to Km values obtained for those in genus Nitrospira. The complete genome of strain CN101 was compared with the genome of two previously sequenced Nitrobacter strains from soil. The genome of strain CN101 had more nitrite/nitrate transporters than that of the other Nitrobacter strains, which implies that the strain CN101 might flexibly adapt to varying nitrite concentrations in soils. Taken together, the physiological and genomic characteristics of the strain CN101 expand knowledge of ecologically important, but understudied genus Nitrobacter.

Session: Terrestrial/Soil/Agriculture 403
Presenter: Jeanette M. Norton
Title: Nitrification communities in maize agroecosystems
Authors: Norton, Jeanette M, Utah State University; Habteselassie, Mussie Y, University of Georgia; Ouyang, Yang, Nutrien Ag Solutions

Several long-term experiments were performed on agricultural sites in Northern Utah, USA and in Watkinsville, Georgia, USA over the last 20 years. The Utah soils are highly calcareous silt loams with a pH of approximately 8.0. The Georgia soils are Cecil sandy loams with initial pH 5.3. We compared the effects of contrasting nitrogen sources of varying quality and quantities on the nitrification process and communities of the ammonia oxidizing bacteria (AOB), ammonia oxidizing archaea (AOA) and nitrite oxidizing bacteria (NOB). Both the abundance and diversity of the AOB were the most changed by fertilization with ammonium sulfate. Process rates and kinetics were also changed by ammonium sulfate applications especially for the AOB while AOA were more responsive to compost amendments. Kinetics of substrate availability and temperature were also investigated. Modeling of nitrification needs to integrate both organism abundance and the kinetic response to be applicable to realistic soil conditions. Metagenomic analysis organic amendments of steer waste compost and poultry litter compost are compared to the soil communities at the respective sites.

Session: Terrestrial/Soil/Agriculture 404
Presenter: Matthew Sima
Title: The feammox process: kinetics, batch incubations, and column studies in the presence of PFAS
Authors: Sima, Matthew, W., Princeton University; Jaffe, Peter, R., Civil Engineering, Princeton University; Huang, Shan, Princeton University

With an increase in both natural and anthropogenic pollutants in terrestrial ecosystems, the need for a strong understanding of how soil biology and nutrient cycling change in the presence of these pollutants is essential. One such pollutant is per or polyfluoroalkyl substances or PFAS which has become ubiquitous in the environment since its discovery in the 1920s. In 2019, the Jaffe lab identified the Acidimicrobium sp Strain A6 as a key bacterium in the Feammox process (ferric ammonium oxidation under anoxic conditions) and in the mineralization of PFAS. This research focuses on understanding the Feammox process in the presence of PFAS first via kinetic modeling and batch scale incubation followed by large scale column experiments. Via a series of batch incubations, PFAS defluorination was shown to scale directly with the Feammox process and with A6 to the 1/m power. This model's robustness has been tested for both different concentrations of PFAS and different concentrations of initial A6 across incubation experiments. However, to better understand the relationship of PFAS and the Feammox process, soil was collected from two PFAS contaminated sites in Lakehurst NJ and Oceana VA and incubated in columns for 50 days under iron rich conditions, Feammox conditions, and DIW conditions. Columns were then disassembled and tested for F-, PFAS, NH4+, and microbial community analysis. Results show that A6 activity can be enhanced in the iron rich condition and the Feammox condition thus leading to higher PFAS defluorination and Feammox rates.

Session: Terrestrial/Soil/Agriculture 405
Presenter: Chiara Smorada
Title: Assessing the impact of PFAS on nitrifying and denitrifying microbial communities in wetland soils

Authors: Smorada, Chiara, M, Princeton University; Huang, Shan, Princeton University; Chen, Chen, Environmental Protection Key Laboratory of Urban Ecological Environment Stimulation and Protection, South China Institute of Environmental Sciences, Ministry of Ecology and Environment; Zhao, Guojun, Environmental Protection Key Laboratory of Urban Ecological Environment Stimulation and Protection, South China Institute of Environmental Sciences, Ministry of Ecology and Environment; Jaffe, Peter, Princeton University

PFAS, a group of persistent organic pollutants, is ubiquitous in the environment and has been observed to affect microbial communities and biochemical cycles. However, the specific impact of PFAS on the stability of microbial communities involved in the nitrogen cycle and nitrogen transformation efficiencies is not well understood. This study aimed to investigate the effects of PFAS on microbial abundance and functional genes related to the nitrogen cycle in soil samples by comparing soil samples contaminated with PFAS to "clean" soils collected from different locations. 16S rRNA sequencing and PCA analysis were used to determine the microbial community diversity under different conditions. Results showed significant differences in the composition of nitrifying and denitrifying microbial communities between samples with and without PFAS. Additionally, a series of batch incubations were conducted with both soil types stimulated with 1ppm PFOA or 1ppm PFOS. After 60 days of aerobic incubation, the addition of PFAS led to a gradual shift in the predominant nitrifiers from AOB (ammonia-oxidizing bacteria) to AOA (archaea ammonia oxidizers), with AOB decreasing from 2.7-5.3% to undetectable levels. Ammonium removal was similar for incubations with and without amended PFOA; however, total nitrogen removal was greater for incubations amended with PFOA. The relative abundance of the main nitrite-oxidizing bacteria, Nitrospira, remained stable, while the proportion of denitrifying bacteria increased from an average of 22.1% to 60.5%. Finally, qPCR analysis revealed an increase in the abundance of the narG and nirK genes and a decrease in other nitrogen functional genes during the incubation.

Session: Terrestrial/Soil/Agriculture 406
Presenter: Daan Speth
Title: Genetic potential for nitrogen cycling metabolism in Denmark
Authors: Speth, Daan, R.[1]; Singleton, Caitlin, M.[2]; Knudsen, Kalinka, S.[2]; Jensen, Thomas, B.N.[2]; Rudkjøbing, Vibeke[2]; Dueholm, Morten, K.D.[2]; Wagner, Michael[1,2]; Nielsen, Per, H.[2]; Albertsen, Mads[2]

1 Center for Microbiology and Environmental Systems Science, Division of Microbial Ecology, University of Vienna
2 Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark

Large-scale metagenomic sequencing efforts have been used to populate the tree of life, and gain insight in the metabolic potential of microorganisms from the human gut to the global oceans. The Microflora Danica project is a unique addition to these large scale datasets, comprising a high resolution sampling campaign of > 10,000 samples taken across a wide range of microbial ecosystems within the country of Denmark. Land use in Denmark is dominated by agriculture (> 75% of land surface), and fertilization using manure or synthetic fertilizer is prevalent (~ 90 kg-N ha-1 yr-1 of each). This leads to high runoff of oxidized nitrogen species, as well as high atmospheric deposition of ammonia throughout the country, suggesting that nitrogen cycle processes are key for microbial communities in all Danish ecosystems. The high resolution sampling of Microflora Danica allows us to investigate the distribution patterns and abundances of the genetic potential for nitrogen cycling processes at an unprecedented scale and to link them to environmental metadata. Using custom databases of nitrogen cycle marker genes, we screen the > 10,000 samples in the Microflora Danica project for the major processes in the microbial nitrogen cycle, with a special emphasis on the key genes for nitrification using diverse organic compounds, including urea, cyanate, and guanidine, as sources of ammonium.

Session: Terrestrial/Soil/Agriculture 407
Presenter: Edi Wipf
Title: Deciphering nitrogen flow networks in grassland biomes: how rhizosphere metabolomes drive nitrifier community dynamics in a Mediterranean climate zone

Authors: Wipf, Edi ML, Lawrence Berkeley National Laboratory; Khasanova, Albina, Lawrence Berkeley National Laboratory; Yuan, Maggie, University of California, Berkeley; Bowen, Benjamin, Lawrence Berkeley National Laboratory; Ceja-Navarro, Javier, Northern Arizona University Pett-Ridge, Jennifer, Lawrence Livermore National Laboratory; Firestone, Mary, University of California, Berkeley; Northen, Trent, Lawrence Berkeley National Laboratory; Zhalnina, Kateryna, Lawrence Berkeley National Laboratory

Grasslands act as global reservoirs of carbon (C) and nitrogen (N) and are crucial for C sequestration under a changing climate. N availability plays a key role in plant C capture and storage in grasslands. Nitrifying archaea, bacteria, and other N-cycling microorganisms, along with plants, regulate soil N availability, C storage, and prevent N loss. Plants produce metabolites that can significantly impact nitrifiers and influence N fate. However, the mechanisms and consequences of plant-nitrifier interactions and the N substrates that drive niche differentiation between ammonia-oxidizing archaea and bacteria remain unknown. In this study, we characterize the microbial community and metabolite dynamics in a Mediterranean annual grassland with urea addition, reduced watering, and across different plant growth stages. Employing archaeal, bacterial, fungal, and microeukaryote community profiling, metabolomics, and network analysis, we sampled across plant development to identify links between rhizosphere metabolites, dynamics of ammonia-oxidizing archaea, bacteria and other N-cycling microbes, and N transformations in soil. We determine that the plant rhizosphere contains numerous N-containing metabolites that initially increase in response to urea addition, which can then be correlated with a changing proliferation of archaea and other N-cycling groups. Interestingly, we further find that two ammonia-oxidizing archaea genera dominate community profiles and are more abundant in bulk soil versus rhizosphere, suggesting potential nutrient competition with the plant mediated by specific classes of plant exudates. These findings highlight the connections between plant development, rhizosphere chemistry, N availability, and the abundance and composition of key N-cycling communities in soil.

Session: Terrestrial/Soil/Agriculture 408
Presenter: Kimura Akari
Title: Diversity of soil microbial community is linked to the stability of ammonia-oxidizing bacteria and archaea community and nitrification rates in a volcanic ash soil

Authors: Akari Kimura, Graduate School of Global Food Resources, Hokkaido University, Japan; Nao Ishige, Graduate School of Global Food Resources, Hokkaido University, Japan Chikae Tatsumi, Department of Biology, Boston University, USA; Yvonne Musavi Madegwa, Alpine Campus, Karlsruhe Institute of Technology, Germany; Eiko Eurya Kuramae, Microbial Community Ecology & Environmental Genomics, Utrecht University; Yoshitaka Uchida, Research Faculty of Agriculture, Hokkaido University, Japan

Repeated application of nitrogen (N) on soils at short intervals is a common agricultural practice, which has a significant impact on the abundance and community of ammonia-oxidizing microbes. Microbial diversity is a key element for the resilience and resistance of ecosystem functions against disturbances. However, the stability of functional microbes such as ammonia-oxidizing bacteria (AOB) and archaea (AOA) has been less investigated, especially under short-interval repeated N fertilizer disturbances. Previous studies have reported a positive correlation between microbial diversity and activity of AOB and AOA. Thus, we hypothesized that a loss of soil microbial diversity has a negative impact on the stability and abundance of ammonia oxidizers under repeated N fertilizer applications. In this study, the dilution-to-extinction approach was used to manipulate soil microbial diversity. Then, the stability and population dynamics of AOB and AOA communities were investigated under repeated N fertilizer applications in different diversity treatments. We found that the AOB and AOA communities were resistant against repeated N disturbance in soil with the highest microbial diversity. For AOB, the community was also resilient in the high diversity treatment. Moreover, the loss of microbial diversity negatively impacted the population growth of AOB following repeated N application. These findings highlight the importance of microbial diversity for the stability of ammonia-oxidizers under short-interval repeated N disturbances.

Session: Terrestrial/Soil/Agriculture 409

Presenter: Jun Zhao

Title: Nutrient acquisition strategies drive coexistence patterns among the dominant lineages of soil ammonia oxidizing archaea

Authors: Zhao, Jun; Huang, Laibin; Chakrabarti, Seemanti; Cooper, Jennifer; Choi, EunKyung; Ganan, Carolina; Tolchinsky, Bryn; Triplett, Eric W.; Daroub, Samira H.; Martens-Habbena, Willm; University of Florida

The evolutionary radiation of terrestrial ammonia-oxidizing archaea (AOA) under the class of Nitrososphaeria is thought to reflect adaptations to a wide range of temperature, pH and other environmental conditions. However, the mechanisms that govern competition and coexistence among Nitrososphaeria lineages in soil remain poorly understood. Here we report a seasonal paired metagenomic, metatranscriptomic, and biogeochemical analysis of nitrifying communities in highly AOA-dominated upland soil. We show that predominant soil Nitrososphaeria compose a patchwork of gene inventory and expression profiles for ammonia, urea and phosphate utilization across 12 major evolutionary lineages commonly found in soil. In contrast, carbon fixation, respiration and ATP synthesis are conserved and expressed consistently among predominant phylotypes, and in situ expression profiles of the encoding gene closely resembled pure culture reference strains under optimal growth conditions. In addition, comparative genomics revealed unique traits that may facilitate the specific adaptation to different environments, such as multiple copies of both high- and low-affinity ammonium transporter genes contained by the lineage specifically adapting to low pH environment, and presence and expression of both V-type and A-type copies of ATPase genes of NS-epsilon lineage which were highly active across a wide range of environmental conditions. Together, these results reveal resource-based coexistence patterns among Nitrososphaeria lineages, and suggest complementary ecophysiological niches associated to differential nutrient and energy acquisition strategies among globally predominant archaeal lineages in soil.

Session: Terrestrial/Soil/Agriculture 410
Presenter: Sakiko Okumoto
Title: Pathways to increase biological nitrification inhibition
Authors: Maharjan, Bal, Texas A&M University, Department of Soil and Crop Sciences; Vitha, Stanislav, Texas A&M University, Microscopy and Imaging Center; Rajan, Nithya, Texas A&M University, Department of Soil and Crop Sciences; Rooney, William, Texas A&M University, Department of Soil and Crop Sciences; Okumoto, Sakiko, Texas A&M University, Department of Soil and Crop Sciences

N fertilizers have been the biggest driver of yield increase in the 20th century. Fertilizer-derived NH4+ is converted to NO2-, then to NO3- in the process termed nitrification. Nitrification and subsequent denitrification (reduction of NO3- and NO2- to N2) both produce N2O as a byproduct, a powerful greenhouse gas (GHG). Due to this fact, the agricultural sector is currently the largest source of anthropogenic N2O pollution. Some plant species are able to suppress nitrification in the root zone through root-exuded chemicals, termed Biological Nitrification Inhibition (BNI), which reduces the loss of N. However, agriculture in 20th century largely ignored BNI, because it was easier to simply add more N fertilizer to compensate for the loss. In light of the urgent need to reduce GHG, however, integrating BNI back into the major crop plants will provide a path to more sustainable agriculture. Sorghum is special among the commonly cultivated crops in that BNI activity is found even in elite varieties. We are interested in increasing the amount of sorgoleone, the major BNI compound in sorghum. In this work, we have analyzed the spatiotemporal expression patterns of sorgoleone biosynthesis enzymes, and discovered that the biosynthesis takes place in the endoplasmic reticulum (ER), on which multiple enzymes form a protein complex. We are also working to build a genomic model to increase BNI secretion in elite sorghum hybrids. The results we obtained will serve as a basis for increasing sorgoleone secretion in sorghum or transferring the ability to other crop plants.

Session: Terrestrial/Soil/Agriculture 411
Presenter: Nithya Rajan
Title: Field-scale Nitrous Oxide Flux from Sorghum in the U.S. Great Plains using Eddy Covariance

Authors: Nithya Rajan[a], Donovan Davis[a], Pramod Pokhrel[b], and Gopal Kakani[c]
[a]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX
[b]U.S.Arid Land Agricultural Research Center, Maricopa, Arizona
[c]Department of Agronomy, University of Florida, Gainesville, FL
Corresponding Author: Nithya Rajan, nithya.rajan@ag.tamu.edu

There is a growing global urgency to address climate change and mitigate greenhouse gas (GHG) emissions. Agriculture plays a crucial role in this effort. Currently, agriculture contributes approximately 10% of GHG emissions in the US, but it also holds the potential to sequester carbon through practices. However, it's important to acknowledge that these practices have a carbon cost. Farm-level life cycle analyses consistently reveal that over 60% of emissions in agriculture originate from the use of nitrogen fertilizers. The release of nitrous oxide (N_2O) from field-applied fertilizers is a highly potent GHG that not only contributes to climate change but also harms the stratospheric ozone layer. To achieve net zero emissions in agriculture, it is crucial to reduce N_2O emissions, especially by decreasing nitrogen fertilizer usage. Additionally, addressing the carbon intensity, which encompasses the emissions of all three major GHGs (CO_2, CH_4, and N_2O) expressed in CO_2 equivalents, while simultaneously ensuring that food production is not negatively affected, is essential. However, the lack of field-scale N_2O emission data poses a challenge in accurately modeling emissions from agricultural fields. In our project, we conducted continuous measurements of GHG emissions at the field level for two years in a 100-acre sorghum field located in the Texas High Plains region. We employed the eddy covariance method to continuously monitor CO_2, N_2O, and CH_4 fluxes. Our findings demonstrate that soil, plant, and environmental conditions strongly influence the diurnal and seasonal exchange of CO_2 and respiration in the field. We also discovered a significant correlation between diurnal N_2O emissions and soil heat flux during periods of high emissions in the growing season. Results from this project will be presented at this conference.

Session: Terrestrial/Soil/Agriculture 412
Presenter: Dimitrios Karpouzas
Title: Synthetic microbial communities of nitrifiers: a tool for ecologically relevant assessment of pesticides toxicity on soil microbes

Kolovou M.[1, 2], Karpouzas D.G.[1], Papadopoulou E. S.[2]
1 Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
2 Laboratory of Environmental Microbiology, Department of Environmental Sciences, University of Thessaly, Larissa, Greece

Single-species tests (SSTs) are used as a conservative Tier I step to determine the toxicity of pesticides on aquatic and terrestrial organisms. Recent studies have identified ammonia-oxidizing microbes (AOM) as indicators of the effects of pesticides, and other pharmaceuticals, on soil microorganisms. In this frame we initially developed and standardized tests for the assessment of pesticides toxicity on phenotypically and ecologically distinct soil AOM, alike to the SSTs used in ecotoxicology. In this study we aimed to build on these SSTs and developed and optimized novel more ecologically relevant *in vitro* ecotoxicity assays based on synthetic microbial communities (SynComs) of nitrifiers composed of ammonia-oxidizing archaea (AOA) and/or bacteria (AOB), and nitrite-oxidizing bacteria (NOB). We have established two SynComs with stable growing patterns upon subculturing: (I) SynCom 1 - *Ca.* Nitrosotalea sinensis (AOA) and *Nitrobacter* sp. NHB1 (NOB) (acidophilic) and (II) SynCom 2 *Nitrosospira multiformis* (AOB) and *Nitrobacter winogradskyi* (NOB) (neutrophilic). Based on our previous results by the SSTs, the established SynComs were used to assess the toxicity of selected pesticides, with first data indicating an increasing pesticide toxicity on SynCom I, while testing on SynCom II is on-going. Further efforts currently focus on the gradual increase of the complexity of our SynComs (e.g. tripartite SynComs with AOB+AOA+NOB, introduction of protists or compartmentalization of nitrification and denitrification processes) will add more ecological value to our testing system. Overall, SynComs of nitrifiers will enable the rapid *in vitro* assessment of the toxicity of pesticides on simple natural assemblages of soil nitrifiers.

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Session: Global Nitrogen Cycling in a warming world 501
Presenter: Jade Bosviel
Title: Spatiotemporal dynamics of Nitrososphaera-driven nitrification and CO2 fixation in a perialpine lake

Authors: Bosviel Jade, Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7B, D-38124 Braunschweig, Germany; Klotz Franziska, Limnological Institute, Department of Biology, University of Konstanz, Universitätsstrasse 10, Konstanz, D-78457, Germany; Kitzinger Katharina, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, Djerassiplatz 1, A-1030 Vienna, Austria; Littmann Sten, Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany; Kuypers Marcel, M.M., Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany; Schleheck David, Limnological Institute, Department of Biology, University of Konstanz, Universitätsstrasse 10, Konstanz, D-78457, Germany; Pester Michael, Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7B, D-38124 Braunschweig, Germany. Technical University of Braunschweig, Institute for Microbiology, Spielmannstrasse 7, D-38106 Braunschweig, Germany.

Large planktonic population of Nitrososphaerota inhabit deep oligotrophic lakes. They are thought to be the key ammonia oxidizers in these freshwater reservoirs and as such responsible for the rate-limiting step in nitrification. In addition, their autotrophic metabolism is hypothesized to influence the carbon budget of these habitats. However, the impact ammonia-oxidizing archaea (AOA) have on nitrogen and carbon cycling in oligotrophic lakes is yet to be quantified. Using 15N-ammonium and 13C-dissolved inorganic carbon (DIC) incubations, we followed spatiotemporal activities of these planktonic AOA in Lake Constance, Germany, which is an important drinking water reservoir for >5 millions of people. Our previous work has shown that AOA are the numerically predominant ammonia oxidizers in this lake. Stable isotope probing revealed that AOA incorporated significantly more 15N-labeled ammonium than most other microorganisms at near-natural conditions and oxidized ammonia at an average rate of 0.22 ± 0.11 fmol cell–1 d–1. Overall ammonia oxidation rates were 7.26 ± 3,36 nmol l–1 d–1 at the center of the hypolimnion (85 m depth) and increased by one order of magnitude just below the thermocline of the lake. Currently, we combine 13C-DIC and 15N-ammonium incubations with selective inhibition of ammonia oxidation to assess the fraction of AOA-driven dark CO2 fixation and ammonium assimilation at different depths. Those experiments will be accompanied by CARD-FISH analyses to quantify AOA and metatranscriptomic analysis. Our final goal is to assess the role AOA play in the overall primary productivity of deep oligotrophic lakes.

Session: Global Nitrogen Cycling in a warming world 502
Presenter: Pearse J. Buchanan
Title: Dynamic oxygen intrusions sustain aerobic nitrite oxidation in anoxic marine zones
Authors: Buchanan, Pearse, J, Carnegie Institute for Science; Sun, Xin, Carnegie Institute for Science; Weissman, J, L, Chapman University; Zakem, Emily, Carnegie Institute for Science

Anoxic marine zones (AMZs) are host to anaerobic metabolisms that drive losses of bioavailable nitrogen from the ocean. Observations of active and abundant nitrite-oxidising bacteria (NOB), long thought to be obligately aerobic and with high oxygen demand, have altered our perception of how nitrogen cycles in these oxygen-deficient waters. Yet, we still lack an explanation for why NOB succeed in AMZs, which inhibits our ability to quantitatively understand and predict their impact on nitrogen loss. The high demand for oxygen by chemoautotrophic NOB suggests that more efficient microbial heterotrophs should win the competition for any oxygen supplied to anoxic zones. Here, we develop a quantitative framework that shows that obligately aerobic NOB can thrive alongside more competitive aerobes in anoxic environments with time-varying intrusions of oxygen, even if the intrusions are relatively infrequent. Ecological theory and modelling suggest that NOB are opportunists that take advantage of fleeting oxygen intrusions to rapidly accumulate biomass. Metagenome-based estimates of higher maximum growth rates for NOB compared to other key aerobic microbial functional types corroborate this hypothesis. Rather than harsh, AMZs with fleeting oxygen intrusions and ample nitrite supply appear as optimal ‘goldilocks’ zones for NOB. The model captures the observed peaks in NOB abundances and activity in anoxic waters. Our results reveal how a time-varying environment facilitates the dynamic coexistence of aerobic and anaerobic metabolisms in anoxic waters and therefore controls nitrogen losses from the ocean.

Session: Global Nitrogen Cycling in a warming world 503
Presenter: Elisa Hernández-Magaña
Title: The role of nitrous oxide (N2O) in the metabolism of ammonia-oxidizing archaea (AOA) under oxygen-depletion

Authors: Hernández-Magaña Elisa, Nordcee, Department of Biology, Faculty of Sciences, University of Southern Denmark; Kraft Beate, Nordcee, Department of Biology, Faculty of Sciences, University of Southern Denmark

Nitrous oxide (N2O) is a greenhouse gas with a warming potential about 300 times higher than CO2 in the atmosphere. Oxygen-depleted ecosystems are important sources of N2O. Most of N2O production in oxygen-depleted ecosystems is attributed to nitrification and/or denitrification. However, other metabolic pathways previously overlooked might require attention when considering N2O production and consumption. N2O has been proposed as intermediate in NO-dismutation, a recently described pathway, which ammonia-oxidizing archaea (AOA) perform upon oxygen depletion. Through this pathway, AOA produce oxygen and dinitrogen as final products with a transient accumulation of N2O. To test the role of N2O in the NO-dismutation pathway, we performed oxygen-depleted incubations with different pure cultures of AOA and combinations of N compounds with different isotopic signature (15NO2- pool + 44N2O spike; 14NO2- pool + 46N2O spike). Then, we evaluated the N2O spike effects on the production of oxygen and on the isotopic signature of N2 and N2O. The experiments confirmed that Nitrosopumilus maritimus and Nitrosopumilus piranensis can reduce N2O to N2. Our results show that N2O is not only an intermediate in archaeal NO-dismutation but that AOA strains can reduce N2O to N2 independently from NO-dismutation. Moreover, the addition of N2O at concentrations higher than the natural accumulation during NO dismutation leads to a cease in oxygen accumulation. We conclude that AOA exposed to oxygen depletion can both, produce and consume N2O. These observations call for a re-evaluation of the role of AOA as sources and sinks of N2O in oxygen depleted ecosystems.

Session: Global Nitrogen Cycling in a warming world 504
Presenter: Kanae Kobayashi
Title: Microbial nitrogen cycling in the deep-sea sediments: combined molecular and stable isotopic analyses to determine the nitrogen flux of nitrification and anammox

Authors: Kobayashi, Kanae, Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC); Makabe, Akiko, Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), (JAMSTEC); Shigemitsu, Masahito, Physical and Chemical Oceanography Research Group, Global Ocean Observation Research Center, (JAMSTEC); Hiraoka, Satoshi, Research Center for Bioscience and Nanoscience (CeBN), (JAMSTEC); Tsuda, Miwako, Project Team for System Development of Marine Environmental Impact Assessment, (JAMSTEC); Miyazaki, Masayuki, Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), (JAMSTEC); Sumida, Tomomi, Research Center for Bioscience and Nanoscience (CeBN), (JAMSTEC); Nomaki, Hidetaka, Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), (JAMSTEC); Nunoura, Takuro, Research Center for Bioscience and Nanoscience (CeBN), (JAMSTEC); Kawagucci, Shinsuke, Marine Biodiversity and Environmental Assessment Research Center (BioEnv), Research Institute for Global Change (RIGC), (JAMSTEC)

Almost 60% of the earth's surface is covered by the deep-sea floor. Quantitative assessment of nitrogen transformations in deep-sea sediments is thus crucial to understand the global nitrogen cycle. Benthic microbial activity and abundance are generally linked with particulate organic carbon flux to the seafloor, related with surface primary production and distance from land. However, the factors regulating microbial activities in deep-sea benthic nitrogen cycles remain uncertain. In this study, we conducted biogeochemical and molecular analyses of sediment columns collected from diverse 12 Northwest Pacific sites, including bathyal trough (Sagami Bay), abyssal plain and hadal trenches (Japan Trench, Izu-Ogasawara Trench). Microbial community composition was characterized using SSU rRNA gene tag sequencing. Concentrations and isotope composition of ammonium, nitrite, and nitrate were determined. Relative abundance of anammox bacteria was high in bathyal trough and hadal trench bottom which presented high TOC concentrations. Nitrifiers were detected in all sites. Relative abundance of ammonium oxidizing archaea was the highest in the abyssal plain which presented the lowest TOC concentration. Enrichment of 15N in ammonium was observed in the surface of sediments in bathyal trough and hadal trench bottom. It might be attributed to isotope effect associated with aerobic and anaerobic ammonium oxidation. The ratio of oxygen to nitrogen isotopic composition of nitrate was 1.7 in the abyssal plain. The relative 18O enrichment in nitrate might be attributed not to kinetic isotope effect but oxygen incorporation during nitrification. Our results demonstrate a wide variation of microbial distribution and isotopic patterns in deep-sea benthic ecosystems.

Session: Global Nitrogen Cycling in a warming world 505
Presenter: Lei Hou
Title: Ocean warming enhances iron use efficiencies of marine ammonia-oxidizing archaea

Authors: Wei Qin, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, OK 73019, USA; Lei Hou, Department of Microbiology and Plant Biology, Institute for Environmental Genomics, University of Oklahoma, Norman, OK 73019, USA; State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361101, China; Min Xu, State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Haikou 570228, China Xiaopeng Brian, Department of Earth Sciences (UCLA), California 90089, USA; Dawn Moran, Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution (WHOI), Woods Hole, Massachusetts 02543, USA; Qian Li, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361101, China; Yue Zheng, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361101, China; Shuh-Ji Kao, State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Haikou 570228, China; State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361101, China; Yao Zhang, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361101, China; Makoto Saito, Department of Marine Chemistry and Geochemistry, WHOI, Woods Hole, Massachusetts 02543, USA; Seth John, Department of Earth Sciences, UCLA, California 90089, USA; Feixue Fu, Marine and Environmental Biology, Department of Biological Sciences, UCLA, California 90089, USA; David A. Hutchins, Marine and Environmental Biology, Department of Biological Sciences, UCLA, California 90089, USA

Ammonia-oxidizing archaea (AOA) are among the most ubiquitous and abundant microorganisms in the ocean. They are almost exclusively responsible for ammonia oxidation in oligotrophic marine environments, thereby altering the bioavailable forms of nitrogen in seawater and impacting primary production and biodiversity. Although key environmental variables such as temperature and trace metal availability are known to influence the growth and activity of marine AOA, there is still only a very limited understanding of how these factors may interact to control their abundance and activity in the rapidly changing ocean. Here, we show that the Fe requirements of a model marine AOA species Nitrosopumilus maritimus SCM1 were highly sensitive to temperature changes. A 5ºC increase in growth temperature reduced its iron requirement by >70%, indicating the strong interactive effects of warming and Fe availability on marine AOA physiology. This decrease in Fe requirement is associated with a substantial increase in iron use efficiencies (IUE, mol C fixed/hr/mol cellular Fe) under Fe-limited and warm temperature conditions, which enables N. maritimus to more efficiently utilize the scarce available Fe supplies to support its growth. We further examined its whole-cell proteomic response to Fe limitation at various temperatures and found that Fe-limited N. maritimus exhibited an enhanced capacity for Fe acquisition and functional replacement of Fe-dense with Fe-free electron carriers, particularly at higher temperatures. These findings suggest that marine AOA may be able to leverage current and future elevated temperatures to better adapt to living in widespread Fe-depleted regions of the ocean.

Session: Global Nitrogen Cycling in a warming world 506
Presenter: Zicheng Su
Title: Comammox Nitrospira dominated as scavenger for urea as nitrogen source
Authors: Su, Zicheng, The University of Queensland; Hu, Zhetai, The University of Queensland; Hua, Zhengshuang, University of Science and Technology of China; Guo, Jianhua, The University of Queensland; Zheng, Min, The University of Queensland

The discovery of comammox added a new process to the nitrogen cycle, where a single microorganism directly converting ammonia to nitrate. Although comammox genomes indicates urea hydrolysis potential, no direct evidence demonstrated their ability to do so. In mixed cultures, heterotrophic microorganisms also encode urease genes, so it is usually unclear whether comammox competes with urea. Previous study enriched comammox from human urine wastewater, indicating urea may be a key factor for selective enrichment. In this study, two reactors were set up for comparative enrichment and operated for >400 days, fed with ammonia and urea at pH 6, respectively. 16S rRNA results showed that Nitrospira became the only nitrifier (up to 12%) in the urea-fed reactor, with metagenomic analysis confirming to be comammox Nitrospira. The affinity of comammox culture for urea was 1.06 ± 0.22 mg urea-N/L, similar to that of the control group (1.18 ± 0.22 mg urea-N/L). The affinity for ammonia was 0.06 ± 0.02 mg NH4+-N/L, parallel to the known comammox strains. Mapped to metagenomic assembled genomes, transcriptomic data showed that the expression of most (>99% of total RPKM) of the amoA, urease, and urea transporter genes was from comammox Nitrospira rather than other species, indicating urea is hydrolyzed and oxidized to nitrate by comammox Nitrospira only. In other words, comammox Nitrospira uses urea alone as nitrogen source for complete nitrification. Our findings suggest that in nitrogen cycling, urea can be directly converted to nitrate by a single microorganism, where comammox Nitrospira plays an important role.

Session: Global Nitrogen Cycling in a warming world 507
Presenter: Xianhui Wan
Title: Pathways of N2O production by marine ammonia-oxidizing archaea determined from dual isotope labeling

Xianhui S. Wan, Princeton University; Lei Hou, University of Oklahoma; Shuh-Ji Kao, Xiamen University; Yao Zhang, Xiamen University; Hua-Xia Sheng, Xiamen University; Hui Shen, Xiamen University; Senwei Tong, Xiamen University; Wei Qin, University of Oklahoma; Bess B. Ward, Princeton University

The ocean is a net source of the greenhouse gas and ozone-depleting substance, nitrous oxide (N2O), to the atmosphere. Most of that N2O is produced as a trace side product during ammonia oxidation, primarily by ammonia-oxidizing archaea (AOA), which numerically dominate the ammonia-oxidizing community in most marine environments. The pathways to N2O production and their kinetics, however, are not completely understood. We use 15N and 18O isotopes to determine the kinetics of N2O production and trace the source of N and O atoms in N2O produced by a model marine AOA species, Nitrosopumilus maritimus. We find that during ammonia oxidation, the apparent half saturation constants of nitrite and N2O production are comparable, suggesting that both processes are enzymatically controlled and tightly coupled at low ammonia concentrations. The constituent atoms in N2O are derived from ammonia, nitrite, O2, and H2O via multiple pathways. Ammonia is the primary source of N atoms in N2O but its contribution varies with ammonia to nitrite ratio. The ratio of 45N2O to 46N2O (i.e., single or double labeled N) varies with substrate ratio, leading to widely varying isotopic signatures in the N2O pool. O2 is the primary source for O atoms. In addition to the previously demonstrated hybrid formation pathway, we found a substantial contribution by hydroxylamine oxidation, while nitrite reduction is an insignificant source of N2O. Our study highlights the power of dual 15N-18O isotope labeling to disentangle N2O production pathways in microbes, with implications for interpretation of pathways and regulation of marine N2O sources.

Session: Global Nitrogen Cycling in a warming world 508
Presenter: Dongyoung Back
Title: The effects of dam gate opening on nitrogen cycling in Nakdong River estuary with an idealized biogeochemical model

Authors: Back, Dongyoung, Virginia Institute of Marine Science, William & Mary, Gloucester Point, VA, USA; Song, Bongkeun, Virginia Institute of Marine Science, William & Mary, Gloucester Point, VA, USA; Harris, Courtney, K., Virginia Institute of Marine Science, William & Mary, Gloucester Point, VA, USA

The Nakdong River Estuary Dam (NRED) was constructed in 1987 to provide multipurpose water to the southeast part of Korea, where millions of people live. Since then, the flow of water has been blocked, and nutrients have been accumulated, resulting in massive blue-green algal blooms every year. The Korean government has proposed dismantling NRED as a restoration project and conducted a field manipulation experiment from June 5 to July 2, 2020, with the opening of the dam gates. However, concerns exist that opening dam gates for extended periods may lead to increase sediment resuspension and benthic nitrogen flux. In particular, the seawater intrusion in the bottom layer greatly influences the nitrogen cycling processes. After the dam gates were opened, noticeable changes in ammonium, nitrite, and nitrate concentrations in the water column and ammonium concentrations in the benthic boundary layer were observed with increases to salinity at locations 6.5 km upstream from the dam. The change rates in ammonium, nitrite, and nitrate concentrations and salinity before and after the opening of dam gates will be used to simulate nitrogen cycling in this area. The Regional Ocean Modeling System (ROMS), including a water column biogeochemistry module, will be used for modeling the effects of the opening of dam gates on the nitrogen flux in the water body. Hereafter, we plan to add a new sediment geochemistry module (i.e., HydroBioSed). This modeling study aims to present the effects of dam removal on benthic nitrogen cycling from long-term perspectives.

Session: Global Nitrogen Cycling in a warming world 509
Presenter: Carolyn Buchwald
Title: Using rate incubations and the stable isotopic composition of nitrate to determine the amount of nitrification, regeneration, and new production during a bloom in the Labrador Sea
Authors: Dempsey, Britton, Dalhousie University; Sipler, Rachel, Bigelow Laboratory for Ocean Sciences; McBride, Colleen, Memorial University of Newfoundland; Buchwald, Carolyn, Dalhousie University

Dissolved inorganic nitrogen (DIN), such as ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-), limits the growth of marine primary production within the euphotic zone, and subsequent export of particulate organic carbon (POC) to depth. Therefore, it is essential to constrain the distribution and magnitude of microbial processes that manipulate DIN and POC pools before assessing the impact of future climatic perturbation on POC export, especially in the climatically important region of the North Atlantic. However, the characterization of intraseasonal dynamics involving these processes within phytoplankton blooms in the Northwest Atlantic is not well defined. Here, we assess the biological controls on DIN speciation and POC production over a 13-day period during the dissipation of a Phaeocystis bloom in the Labrador Sea using measured rates of ammonium, nitrite, and nitrate uptake, ammonium regeneration, and nitrification using ^{15}N tracer incubations, and natural abundance $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ measurements. Measured rates of nitrogen transformation processes are then used to constrain a 1-D reaction-diffusion model of ammonium, nitrite, nitrate, $\delta^{15}N_{NO3}$, and $\delta^{18}O_{NO3}$, where associated isotope effects of each process can be inversely determined. Results from this work will: (i) improve our understanding of the expression of nitrification and uptake within $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ profiles, (ii) and help reassess classical assumptions related to POC export in the open ocean by examining the vertical distribution of new production, regeneration, and nitrification.

Session: Global Nitrogen Cycling in a warming world 510
Presenter: Jessica Bullington
Title: Distribution and diversity of nitrifying microorganisms within the subterranean estuary at Stinson Beach, California

Authors: Bullington, Jessica, A, Stanford University, Department of Earth System Science; Langenfeld, Kathryn, Stanford University, Department of Civil and Environmental Engineering; Phaneuf, Jacob, R, Stanford University, Department of Civil and Environmental Engineering, Boehm; Alexandria, B, Stanford University, Department of Civil and Environmental Engineering; Francis, Christopher, A, Stanford University, Department of Earth System Science

Sea level rise will likely affect the flux of nitrogen from terrestrial groundwater to the ocean. The magnitude of this flux is mediated by microorganisms living in beach aquifers. Subterranean estuaries within beach aquifers have tidally-dynamic and steep physicochemical gradients (<10 m) providing a unique lens into the structuring forces on microbial community ecology, physiology, and metabolic activity. However, there are relatively few publications on the microbiology of subterranean estuaries, particularly targeting a comprehensive understanding of nitrogen cycling. Previous research at Huntington Beach, CA found a dramatic shift in the relative proportion of ammonia-oxidizing bacteria (AOB) versus archaea (AOA) along the salinity gradient. The biogeochemical implications for this pattern remain unclear, especially in the context of sea level rise. In this study, we explored the microbial ecology of the subterranean estuary at Stinson Beach, CA over two neap-to-spring tidal transitions in winter and early fall of 2022 using qPCR and 16S rRNA gene amplicon sequencing. There were notably high inorganic nitrogen concentrations in both seasons with corresponding distributions of diverse AOA, AOB, nitrite-oxidizing bacteria (NOB), and anammox bacteria. Notably, AOA were consistently dominant, exceeding 15% of the total microbial community in certain conditions. Overall, salinity was a more important determinant of variation in abundance of AOA, AOB, and NOB than tidal or seasonal variation alone. This research provides new insights into the spatiotemporal dynamics of nitrifier communities in these important yet understudied coastal systems, which will be complemented with meta-omic and cultivation-based analyses.

Session: Global Nitrogen Cycling in a warming world 511
Presenter: Paula García-Otero
Title: Ecology and physiology of ammonia-oxidizing archaea under sulfidic conditions
Authors: García-Otero, Paula, University of Southern Denmark; Oo Yee, Mon, University of Southern Denmark; Kraft, Beate, University of Southern Denmark

The ammonia-oxidizing archaeon (AOA) Nitrosopumilus maritimus SCM1 has recently been shown to produce oxygen, when exposed to oxygen depletion. A potential explanation for the frequent occurrence of AOA in oxygen-depleted environments would be, that they can perform this proposed pathway of oxygen production, NO-dismutation. The aim of this work is to study the ecology of AOA in the stratified water column of the Mariager Fjord, an euxinic basin in Denmark. Surprisingly, amoA gene copy numbers peaked in sulfidic depths adjacent to the chemocline. 16S rRNA gene amplicon sequencing showed that one sequence variant that affiliated with Nitrosopumilus dominated the archaeal community in and below the chemocline. CARD-FISH and Bioorthogonal Noncanonical Amino Acid Tagging (BONCAT) demonstrated that AOA related to Nitrosopumilus were active by synthesizing new proteins during anoxic incubation experiments. The metabolic pathways and activity of these AOA are being evaluated by metagenomic analyses. To explore the influence of sulfide on the physiology of AOA in more detail, ammonia oxidation activity of Nitrosopumilus maritimus SCM1 was measured at sulfide concentrations ranging from 0 to 100µM and at oxygen concentrations of 2 µM. N. maritimus oxidized ammonia under initial sulfide concentrations of up to 20µM but at reduced rates in comparison to incubations without sulfide. Higher Sulfide concentrations completely inhibited ammonia oxidation activity. All in all, we show that AOA are not limited to the chemocline of anoxic basins but can remain abundant and active at low sulfide concentrations.

Session: Global Nitrogen Cycling in a warming world 512
Presenter: Naomi Intrator
Title: Nitrite Reductase gene (nirK) Phylogeny and Biogeography
Authors: Intrator, Naomi, Jayakumar, Amal; Lin, Mandy; Ward, Bess, B, Princeton University, Princeton, NJ, USA

Nitrite reduction, encoded by the gene nirK, is an essential step in the oceanic Nitrogen cycle especially in the process of denitrification. However, the nirK gene is not exclusive to denitrifiers but is also found in nitrifiers, ammonia- and nitrite-oxidizing bacteria (AOB and NOB) as well as ammonia-oxidizing archaea (AOA). nirK is found throughout the ocean, including in oxygenated surface water as well as in oxygen minimum zones (OMZs). nirS, the gene for the iron type nitrite reduction, is found in some denitrifying organisms and is also abundant in the ocean but is apparently less diverse than nirK. The diverse and complex evolutionary history of the nirK genes makes it challenging to study the population structure and distribution of nirK containing organisms in the environment. Several distinct phylotypes of nirK occur between and throughout OMZs, suggesting significant phylogenetic and functional diversity of denitrifying organisms. Using sequences collected from public databases, we surveyed the phylogeny and global biogeography of nirK. Sequences were clustered into operational taxonomic units (OTUs) based on DNA sequence identity. The OTUs that contained sequences collected from aquatic isolation sources (~35% of all OTUs), representing over 4,000 sequences, were analyzed further. OTUs were further grouped into archetypes based on representative regions of the gene. Further analysis of the isolation sources and environmental distribution of the sequences showed strong biogeographical separation among archetypes, providing a clearer picture of how the phylogeny of nirK containing organisms are distributed across diverse marine environments.

Session:	Global Nitrogen Cycling in a warming world	513
Presenter:	Clarissa Karthäuser	
Title:	Investigating nitrogen cycling in marine particles while keeping them afloat	
Authors:	Karthäuser, Clarissa; Fucile, Paul; Martocello, Donald; Wankel, Scott; Buesseler, Ken; Sievert, Stefan; Woods Hole Oceanographic Institution	

Marine snow particles and fecal pellets carry organic matter seeded with microbial communities from the productive sunlit surface ocean into the deeper water layers. As these particles provide concentrated organic carbon and nutrients and a platform for organisms to grow on and attach to, they are likely to be hotspots of both heterotrophic and autotrophic microbial activity. Recent studies suggest that particles can harbor a range of aerobic to anaerobic processes, as high respiration activity inside the particle can create anoxic micro-niches and steep nutrient gradients. Specifically, denitrification and nitrification have been predicted to occur associated with particles based on modeling, experimental data, and functional gene analysis. Yet, studies that quantify these processes and identify niches and interactions between microbial players in the individual particle ecosystem are currently lacking. To change this, I teamed up with an engineer to build a new incubator, which allows the simultaneous incubation of sixty individual particles while quantifying respiratory and autotrophic processes. This non-destructive approach enables us to further characterize each incubated particle and link this information with particle properties such as size, sinking velocity and elemental composition. My poster at the upcoming ICON meeting will showcase our innovative technique and provide preliminary results from incubating marine particles with stable isotope tracers under aerobic conditions. This approach enables us to gain insights into the intricate dynamics of nitrogen cycling associated with marine particles and the resulting pathways of organic matter degradation, new carbon fixation and release of various products.

Session: Global Nitrogen Cycling in a warming world 514
Presenter: Akiko Makabe
Title: Nitrification rates in the eastern Indian Ocean
Authors: Makabe, Akiko, Japan Agency for Marine-Earth Science and Technology (JAMSTEC); Yoshikawa, Chisato, Japan Agency for Marine-Earth Science and Technology (JAMSTEC); Kotajima, Syotoku, Tokyo University of Agriculture and Technology
Yokokawa, Taichi, (JAMSTEC); Nunoura, Takuro, (JAMSTEC); Koba, Keisuke, Kyoto University

Nitrification is ubiquitous in the ocean and plays an important role as a producer of nutrients for primary producers, a primary producer in deep sea by fixation of carbon dioxide, and a producer of nitrous oxide which is one of the greenhouse gases. It has been reported that ammonia-oxidizing archaea has a potential activity to use urea as a substrate of nitrification. It is important to reveal how much nitrification rates using both ammonia and urea are in the ocean. We conducted on-board incubation experiments during a cruise MR15-05 of R/V Mirai in the eastern Indian Ocean. Water samples were collected from Niskin samplers into glass vials directly without air. Vials were stored at in-situ water temperature after addition of nitrogen-15 enrichment ammonium or urea. We measured excesses of nitrogen-15 in NOx, and then nitrification rates were calculated. Our results indicated that nitrification activities using urea as substrate were comparable to that using ammonium in the open ocean. We will present vertical profiles of nitrification rates from ammonium and urea as a substrate in the eastern Indian Ocean.

Session: Global Nitrogen Cycling in a warming world 515
Presenter: John Tracey
Title: All about nitrite: Exploring nitrite sources and sinks in the Eastern Tropical North Pacific Oxygen Minimum Zone

Authors: Tracey, J., Columbia University, NY, USA; Babbin, Andrew R., MIT, Cambridge, MA, USA; Wallace, Elizabeth R., Princeton University, Princeton, NJ, USA; Sun, Xin, Carnigie Institution for Science, Stanford, CA, USA; DuRussel, Katherine L., Northwestern University, Evanston, IL, USA; Frey, Claudia, Universitiy of Basel, Switzerland; Martocello III, Donald E., MIT, Cambridge, MA, USA; Tamasi, Tyler, MIT, Cambridge, MA, USA; Oleynik, Sergey, Princeton University, Princeton, NJ, USA; Ward, Bess B., Princeton University, Princeton, NJ, USA

Oxygen minimum zones (OMZs), due to their large volumes of perennially deoxygenated waters, are critical regions for understanding how the interplay between anaerobic and aerobic nitrogen (N) cycling microbial pathways affects the marine N budget. Here we present a suite of measurements of the most significant OMZ N cycling rates, which all involve nitrite (NO_2^-) as a product, reactant, or intermediate, in the Eastern Tropical North Pacific (ETNP) OMZ. These measurements and comparisons to data from previously published OMZ cruises present additional evidence that NO_3^- reduction is the predominant OMZ N flux, followed by NO_2^- oxidation back to NO_3^-. The combined rates of both of these N recycling processes were observed to be much greater (up to nearly 200x) than the combined rates of the N loss processes of anammox and denitrification, especially in waters near the anoxic / oxic interface. We also show that NO_2^- oxidation can occur when O_2 is maintained near 1 nM by a continuous purge system, NO_2^- oxidation and O_2 measurements that further strengthen the case for truly anaerobic NO_2^- oxidation. We also evaluate the possibility that NO_2^- dismutation provides the oxidative power for anaerobic NO_2^- oxidation. The partitioning of N loss between anammox and denitrification differed widely from stoichiometric predictions of at most 29% anammox; in fact, N loss rates at many depths were entirely due to anammox. Our new NO_3^- reduction, NO_2^- oxidation, dismutation, and N loss data shed light on many open questions in OMZ N cycling research, especially the possibility of truly anaerobic NO_2^- oxidation.

Session: Global Nitrogen Cycling in a warming world 516

Presenter: Zihuizhong Ye

Title: Anthropogenic impact on nitrifying and denitrifying microbial communities in coastal zone

Authors: Chen, Chen, State Environmental Protection Key Laboratory of Urban Ecological Environment Simulation and Protection, South China Institute of Environmental Sciences, Ministry of Ecology and Environment, Guangzhou, China, 510535; Ye, Zihuizhong, Department of Civil and Environmental Engineering, Princeton University, Princeton, New Jersey, USA, 08540; Wang, Yuji, State Environmental Protection Key Laboratory of Urban Ecological Environment Simulation and Protection, South China Institute of Environmental Sciences, Ministry of Ecology and Environment, Guangzhou, China, 510535; Huang, Shan, Department of Civil and Environmental Engineering, Princeton University, Princeton, New Jersey, USA, 08540

The coastal zones are under significant stress due to human activities. However, different terrestrial land-use types introduce varying anthropogenic pollution sources into these coastal areas, then affecting the nitrifying and denitrifying microbial communities in aquatic ecosystems. This study focused on three representative coastal zones in Guangdong, China, with distinct primary anthropogenic sources. 18 sampling locations were selected to collect physicochemical and microbial information. The Dayawan Sea region (HZ) represents coastal zones surrounded by industrial pollutants. The Huangmao Sea region (ZH) is an estuarine area affected by urban pollution. The Zhangjiang Sea (ZJ) receives agricultural contaminants. The results revealed significant differences in the alpha and beta diversity indices ($p < 0.05$) between the nitrifying and denitrifying communities in samples influenced by different anthropogenic sources. The ZH estuary, influenced by urban sources, exhibited the highest abundance of ammonium oxidizing archaea (AOA) at 32.6%, with Nitrosopumilaceae being the dominant family (>99% AOA). Similarly, ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), and Feammox bacteria (involved in ammonium oxidation coupled with iron reduction) also showed significantly higher numbers in ZH compared to HZ and ZJ. Conversely, high numbers of denitrifiers were observed in ZJ, the agricultural and maricultural sea region. Canonical Correspondence Analysis (CCA) and Mantel's test demonstrated that the composition of microbial communities was influenced by nitrogen (N) and phosphorus (P), suggesting that nutrients play a crucial role in stimulating nitrifiers. Network analysis further indicated that the urban estuary enhanced interactions among the nitrifying and denitrifying microbial communities, thereby promoting biochemical processes.

Session: Global Nitrogen Cycling in a warming world 517
Presenter: Chisato Yoshikawa
Title: Development of a marine ecosystem model for impact assessment of acidification on nitrification
Authors: Yoshikawa, Chisato, JAMSTEC; Makabe, Akiko, JAMSTEC; Sasai, Yoshikazu, JAMSTEC

Ocean acidification impacts on various marine organisms and biogeochemical processes. Incubation experiments suggest that ocean acidification could reduce nitrification rates and could increase oceanic N2O production during nitrification. The response of marine microbial activities to ocean acidification might play a crucial role in the future evolution of air–sea fluxes of greenhouse gases such as CO2 and N2O. This study developed a 1D marine ecosystem model incorporating nitrification with pH-dependent terms and N2O productions by ammonium oxidation and nitrite reduction. We applied this model to the JAMSTEC time-series subarctic and subtropical sites (K2 and S1) in the western North Pacific. The model was validated with observed nitrogen concentration and successfully simulated the higher nitrification rates, the higher N2O concentration, and the higher N2O production rates at K2 compared with S1. The annual mean N2O emission fluxes were estimated to be 42 mgN m^{-2} yr^{-1} at K2 and 3 mgN m^{-2} yr^{-1} at S1. The N2O was mainly produced by ammonium oxidation at K2 and was produced by both ammonium oxidation and nitrite reduction at S1. In the case study, the reduction of pH by 0.4 decreases the nitrification rate by 10-20 nmol/L/day in the high-nitrification depth around the bottom of the mixed layer in June and July at K2. In this presentation, we will also discuss the impact of ocean acidification on oceanic N2O production during nitrification.

Session: Symbiosis/Interactions 601
Presenter: Sarah Al-Ajeel
Title: Competition versus cooperation: Ecological strategies of nitrifiers
Authors: Al-Ajeel, Sarah; Sakoula, Dimitra; Geerlings, Nicole; Palatinszky, Márton; Pejvac, Petra; Heldwein, Nathalie; Daims, Holger, Department of Microbial Ecology, University of Vienna, Austria

Nitrification is an important process in engineered wastewater treatment systems, as it prevents high levels of ammonia and nitrite from being released into aquatic environments. In water treatment, ammonia oxidizing bacteria and archaea (AOB and AOA), nitrite oxidizing bacteria (NOB), and complete ammonia oxidizers (comammox Nitrospira) can be responsible for nitrification. Comammox Nitrospira are thought to be yield strategists that maximize ATP production per substrate consumed, whereas canonical nitrifiers are rate strategists that maximize ATP production per time unit (Costa et al. 2006). Theoretical predictions from individual-based mathematical simulations have shown that yield strategists will outcompete rate strategists in environments with limited substrate flux (Kreft, 2004). This is particularly relevant in substrate-limited flocs and biofilms, a frequent growth morphology for nitrifiers in water treatment systems. So far, this theoretical model has not been experimentally validated and a nitrifying community is an ideal system to study these ecological trade-offs. Here, we will experimentally test the rate versus yield trade-off in nitrifying communities by investigating the effect of ammonium concentration on the composition and spatial structure of nitrifying flocs over time. Spatial and physiological data will be combined with an individual-based modelling approach to study the effect of ammonium flux on the microbial composition. Flocs from synthetic nitrifier communities will be analyzed for relative abundance and spatial distribution using quantitative FISH and image analysis. Possible interactions of nitrifier community aggregates as a function of ammonium loading will be assessed.

Session: Symbiosis/Interactions 602
Presenter: Nicole M. J. Geerlings
Title: From the single cell to the community: metabolic heterogeneity in comammox Nitrospira
Authors: Geerlings, Nicole M.J.; Al-Ajeel, Sarah; Pjevac, Petra; Kitzinger, Katharina; Daims, Holger, Division of Microbial Ecology, University of Vienna

For a long time nitrification was thought to be a two-step process mediated by two distinct functional groups of microorganisms, although the existence of microbes capable of complete ammonia oxidation (comammox) was predicted to exist in oligotrophic environments. In 2015, Nitrospira bacteria performing complete ammonia oxidation were discovered in enrichment cultures retrieved from biofilms. Thus far, the behavior of an individual comammox cell has been inferred from the behavior of the population. It is assumed that each cell performs complete ammonia oxidation and grows at an average rate. However, the rates at which different metabolic activities are performed by genetically identical cells may vary within populations. For comammox, metabolic heterogeneity is expected, but it is unknown to what extent this occurs and how it depends on factors such as substrate availability. Theoretically, metabolic heterogeneity might go as far as the development of a division of labor within a comammox community, where one subpopulation oxidizes ammonia to nitrite that is oxidized to nitrate by another subpopulation. Here, we show the experimental set-up we will use to analyze the metabolic heterogeneity and potential division of labor within a comammox community. This includes the use of cutting-edge stable isotope probing, molecular and chemical imaging techniques to uncover the physiology of comammox Nitrospira at the single cell level. We will show the first preliminary results that provide insights into the single-cell behavior of comammox and elucidate how collective properties of microbes arise from metabolic interactions happening at the microscale.

Session: Symbiosis/Interactions 603

Presenter: Grace Kennedy

Title: Friends with benefits? Exploration of symbiotic ammonia-oxidizing archaea within ascidian tunic microbiomes

Authors: Kennedy, Grace; Tolar, Bradley, B, Department of Biology and Marine Biology, University of North Carolina Wilmington

Ammonia-oxidizing archaea (AOA) are considered ubiquitous in marine environments, where they contribute substantially to global nitrogen cycling via nitrification. Yet, little is known regarding symbiotic AOA within marine invertebrate microbiomes. Though most host-associated archaeal studies have focused on marine sponges thus far, molecular surveys have also identified AOA in ascidian microbiomes. Ascidians – more commonly known as sea squirts – are sessile filter feeders that are often found attached to docks and harbors, similar to sponges. Due to increased globalization and vessel activity, both non-native and native ascidian species inhabit and thrive in a wide range of coastal environments, including those with elevated nutrient loading. This project aims to investigate host-associated AOA inhabiting marine ascidian invertebrates along coastal North Carolina near Wilmington. Microbiomes were sampled from ascidian tunic tissue to determine AOA abundance and diversity. These results were compared to previous studies that suggest AOA may be dominant within microbiomes of specific species of ascidians. It is hypothesized that AOA could provide numerous advantages for invertebrate hosts, including nitrogen waste removal, detoxification of excess nitrogen, and nutrition (including vitamins). In return, AOA may receive light protection and/or nutrients from the host. Further host-associated AOA studies are necessary to determine implications on biogeochemical cycling and their contributions to system-level nitrification, as research thus far has focused on free-living AOA in coastal systems. Understanding any potential health advantages provided to marine organisms by AOA will be critical to predict organismal responses to changing climate and ecological stressors.

Session: Symbiosis/Interactions 604
Presenter: Madison P. Kimbrel
Title: Oxidative stress alters the heterotrophic community structure of ammonia-oxidizing enrichment cultures
Authors: Kimbrel, Madisen, P.; Spencer, Madelynn, D.; Bollmann, Annette, Miami University of Ohio

Ammonia-oxidizing bacteria (AOB) are important contributors to the global nitrogen cycle, carrying out the first step of nitrification. They are found in freshwater lakes and wastewater treatment plants, where they live in communities with heterotrophic microorganisms. Bacteria living in aquatic environments are frequently exposed to oxidative stress, from metabolic byproducts or photochemical processes. Oxidative stress can damage DNA, lipids, and proteins of bacteria. Some bacteria possess defense mechanisms to protect themselves against oxidative stress, like superoxide dismutase, catalase, and peroxidase. A previous proteomics study showed that the presence of heterotrophic bacteria reduces the effects of oxidative stress on AOB (Sedlacek et al., 2016). Here we investigated the effects of hydrogen peroxide (H2O2) exposure on the heterotrophic community of two AOB enrichment cultures. The cultures originated from a freshwater eutrophic lake in Ohio, USA, and received varying exposure to different concentrations of H2O2 for up to six growth cycles. Growth and activity of AOB was monitored by measuring nitrite production and the bacterial community composition was determined by 16S rRNA gene amplicon sequencing. The most drastic changes in community structure were observed when the enrichments were incubated at 0.1 mM H2O2. The relative abundance of multiple taxa was significantly different at 0.1 mM H2O2 compared to the control and the incubations at 0.01 mM H2O2. Intermittent incubation with H2O2 resulted in different bacterial communities compared to control and constant exposure. This study provides insight into heterotrophic bacteria that might provide support to AOB under oxidative stress.

Session: Symbiosis/Interactions 605
Presenter: Sabita Ghimire
Title: Competition for ammonium between a complete ammonia oxidizer and an ammonia-oxidizing archaea from a freshwater lake in Ohio

Authors: Sabita Ghimire*, Matt E. Weaver Jr*, Madisen P. Kimbrel and Annette Bollmann, Miami University of Ohio

Complete ammonia oxidizers (comammox) and ammonia-oxidizing archaea (AOA) are co-existing in many different environments. Most members of both groups have a high affinity for ammonium. Therefore, it is difficult to predict which group would prevail if ammonium would become limiting in the environment. We investigated the competition between a comammox and an AOA under limiting and elevated ammonium concentrations. Cmx-BO4 is a monoclonal enrichment of comammox *Nitrospira* sp. BO4. AOA-BO1 is an enrichment of AOA *Nitrosoarchaeum* sp. and nitrite oxidizing bacteria *Nitrospira* sp. BO1. Both ammonia oxidizers were enriched from the sediment of Lake Burr Oak in eastern Ohio. Competition experiment for limiting ammonium were conducted in chemostats, while the impact of elevated ammonium was tested in batch cultures. The abundance of the ammonia oxidizers was followed by qPCR. When ammonium was limiting, *Nitrospira* sp. BO4 dominated the co-culture over time while the abundance of *Nitrosoarchaeum* sp. BO1 decreased. The abundance of the nitrite oxidizer *Nitrospira* sp. BO1 also decreased to a new equilibrium with *Nitrospira* sp. BO4. In the presence of 0.05- and 0.5 mM ammonium simulating elevated ammonium concentrations also, *Nitrospira* sp. BO4 became dominant in the mixed cultures. Nitrite-oxidizing bacteria *Nitrospira* sp. BO1 also decreased in the mixed cultures and the ratio of *Nitrospira* sp. BO1 to *Nitrospira* sp. BO4 was smaller than in chemostats indicating a better supply of nitrite in chemostats compared to batch cultivation. The reduction in abundance of *Nitrospira* sp. BO1 in mixed culture could be explained by the competition for nitrite between comammox and nitrite-oxidizing bacteria. Overall, the results show that the freshwater comammox strain *Nitrospira* sp. BO4 is able to grow better and subsequently outcompete the AOA strain *Nitrosoarchaeum* sp. BO1 under the tested conditions of limiting and elevated ammonium. The ability of *Nitrospira* sp. BO4 to outcompete *Nitrosoarchaeum* sp. BO1 could be explained by the higher yield from ammonia-oxidation and more efficient carbon fixation pathway of comammox compared to AOA. This observation is supported by the 3.5 times higher abundance of comammox compared to AOA in the sediment of Lake Burr Oak.

Session: Functional Omics 701
Presenter: Samantha Fortin
Title: Novel nitrite oxidizing bacteria perform nitrite oxidation in oxygen minimum zones

Authors: Fortin, Samantha, G, Department of Geosciences, Princeton University
Sun, Xin, Department of Global Ecology, Carnegie Institution; Jayakumar, Amal, Department of Geosciences, Princeton University; Ward, Bess, B, Department of Geosciences, Princeton University

High rates of nitrite oxidation have been found in the anoxic core of oxygen minimum zones (OMZs) despite the requirement for oxygen and the obligate aerobic metabolism of all known nitrite oxidizers. Metagenomic sequences were obtained from the Eastern Tropical North Pacific (ETNP) and Arabian Sea OMZs from depths including the oxic surface waters, oxycline, and anoxic core. Metagenome assembled genomes (MAGs) of ten Nitrospinae-like nitrite oxidizing bacteria (NOB) "species" were obtained from the metagenomic sequences, eight of which were unrelated to any previously identified NOB. These OMZ NOB MAGs were present only in the OMZ regions, not in any other open ocean biome, and NOB previously identified from oxic systems were not detected in oxygen deficient OMZ samples, indicating strong niche separation in NOB communities. The strong correlations between the relative abundance and distribution of the OMZ NOB MAGs, OMZ NOB MAG nitrite oxidoreductase expression and abundance, and nitrite oxidation rates imply that the novel OMZ NOB MAGs are responsible for nitrite oxidation in OMZs. OMZ NOB MAGs contain genes consistent with a micro-aerophilic metabolism, though also include genes, many with unknown functions, not present in NOB isolated from oxic waters. Overall, these potentially aerobic OMZ NOB have adapted to the OMZ niche where they dominate the NOB community and are responsible for the high rates of observed nitrite oxidation.

Session: Functional Omics 702
Presenter: Birch Lazo-Murphy
Title: Transcriptomic analysis of the nitrous oxide-producing salt marsh sediment fungi, Purpureocillium lilacinum
Authors: Lazo-Murphy, Birch, M, University of South Carolina; Peng, Xuefeng, University of South Carolina

The emission of the greenhouse gas nitrous oxide has been increasing from salt marsh environments. While bacterial denitrification is considered the largest source of nitrous oxide production from salt marshes, little focus has been placed on the role of salt marsh sediment fungi – a small portion of the salt marsh sediment microbiome which may disproportionately contribute to nitrous oxide emissions as they lack the gene required to convert nitrous oxide to dinitrogen gas. We measured nitrous oxide production rates and performed transcriptomic analysis using a Purpureocillium lilacinum strain isolated from salt marsh sediments, which was cultivated under different oxygen (anoxic vs. oxic), carbon (Spartina stem vs. glucose), and nitrogen (10 vs. 100 µM nitrite) conditions. The fungal nitrite reductase (nirK) and nitric oxide reductase (p450nor) was upregulated under anoxic (sulfidic) conditions compared to oxic conditions, supporting the hypothesis that salt marsh sediment fungi produce nitrous oxide via denitrification, even under sulfidic conditions. P. lilacinum upregulated p450nor at high nitrite concentrations (100 µM), consistent with the higher nitrous oxide production rate (47.7 nmol/day) at 100 µM nitrite than at 10 µM nitrite (3.5 nmol/day). Under both oxic and anoxic conditions, P. lilacinum expressed cellulase, hemicellulase, and pectinase genes responsible for breaking down plant cell walls (e.g. GH5, GH43, GH45, and GH28) when cultivated with Spartina stems. Our findings imply a previously overlooked role of salt marsh sediment fungi in nitrous oxide production and lignocellulosic biomass turnover.

Session: Functional Omics 703
Presenter: Christopher Sedlacek
Title: The role, or lack thereof, of sNOR during denitrification by ammonia-oxidizing bacteria

Authors: Loy, Daniel, Centre for Microbiology and Environmental Systems Science, University of Vienna; Herbold, Craig School of Biological Sciences, University of Canterbury Giguere, Andrew Centre for Microbiology and Environmental Systems Science, University of Vienna; Daims, Holger Centre for Microbiology and Environmental Systems Science, University of Vienna; Wagner, Michael Centre for Microbiology and Environmental Systems Science, University of Vienna; Pjevac, Petra Centre for Microbiology and Environmental Systems Science, University of Vienna; Sedlacek, Christopher Centre for Microbiology and Environmental Systems Science, University of Vienna

Ammonia-oxidizing bacteria (AOB) perform not only the first step of nitrification, but also the partial denitrification of nitrite (NO2-) to nitric (NO) or nitrous oxide (N2O) under low-oxygen or anoxic conditions. The genetic inventory required for the partial denitrification of NO2- in microorganisms is well established, with a sequential reduction of NO2- to NO by nitrite reductases (NIRs) and then to N2O by nitric oxide reductases (NORs). Only one type of NIR has been identified in AOB, nirK. However, two potential NOR enzymes have been identified, the well characterized cNOR (norCBQD) and the uncharacterized sNOR (norSY-senc-orf1). A survey of more than 80 genomes derived from cultured AOB confirmed that the sNOR is indeed widespread in AOB and occurs in genomes as both the only annotated NOR and in combination with cNOR. However, it is unclear whether the sNOR in fact contributes to N2O production during denitrification. Here, we investigated the anoxic NO and N2O production profiles of three AOB species (Nitrosomonas europaea Nm50, Nitrosospira briensis Nsp1, and Nitrosospira briensis Nsp12) encoding different combinations of cNOR and sNOR. A combination of microsensors and an oxygen optode were used to simultaneously measure O2, NO, and N2O in real time for up to several days. The results clearly show that the only AOB tested that was indeed able to enzymatically produce N2O encoded a cNOR. The presence of a sNOR alone did not confer the ability for AOB to enzymatically produce N2O from NO.

Session: Functional Omics 704

Presenter: Olabisi Adebisi

Title: The ecophysiological relevance of low salinity in niche differentiation of ammonia oxidizing microorganisms

Authors: Adebisi, Olabisi O.; Lee, Ui-JU; Gwak, Joo-Han; Abiola, Christiana; Si, Ok-Ja; Rhee, Sung-Keun,* Department of Biological Sciences and Biotechnology, Chungbuk National University, Chungdae-ro, Seowon-Gu, Cheongju 28644, Republic of Korea

Nitrification, the sequential aerobic oxidation of reduced nitrogen compounds (primarily ammonia) to nitrate via nitrite, is considered pivotal in the global biogeochemical nitrogen cycle. This process is microbially executed by three different groups of ammonia-oxidizing microorganisms (AOM), i.e., ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and complete ammonia oxidizer (comammox). Freshwater is an important source of water for living things on Earth. Most freshwater lakes and groundwater have low salinity values with electrical conductivity (EC) typically less than 150 µS/cm. The population kinetics and nitrifying activities of AOM in such an environment with low-salinity conditions are not fully understood. Here, we report the effect of the salinity of media with EC ranging from 71 µS/cm to 2700 µS/cm on the nitrification activity of AOM. Low salinity had no significant effect on the growth of AOB and comammox, however, it negatively influenced only AOA's growth and nitrifying activities. The effect is lesser in AOA group 1.1b (Nitrososphaera viennensis EN76, Nitrosocosmicus oleophilus MY3) compared to 1.1a (Nitrosotenuis chungbukensis MY2, Nitrosoarchaeaum koreensis MY1) AOA. Transcriptomic and proteomics analysis reveals that proteins associated with cell homeostasis and membrane physiology are differentially expressed in cells of strain MY2 subjected to low salinity conditions. Thus, the variation observed in the sensitivity of AOA to low salinity might be due to their differences in cell structure and physiology, and their particularities in adaptation to changes in environmental osmolarity. Conclusively, findings from this study have enlightened us on the niche segregation attribute of low salinity on nitrifiers.

Session: Functional Omics 705
Presenter: Clair E. Elbon
Title: Autoflourescent Marinobacter dominate long term marine enrichments grown on methane and nitrate

Authors: Elbon, Claire, E, Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA; Glass, Jennifer, B, Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA Stewart, Frank, J, Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT

Microbial cycling of greenhouse gases (GHGs) may reduce or amplify the effects of climate change. Anaerobic hydrocarbon oxidation coupled to nitrate/nitrite reduction is a known microbial metabolism cycling GHGs, but end-product formation of nitrous oxide, as well as the identity of microbes capable of this metabolism remains unknown and understudied. We explored these questions with combined metagenomic/-transcriptomic surveys of oxygen deficient zones (ODZs), and identification of microbes coupled to metabolic activity in multi-year anoxic enrichment cultures collected from the Eastern Tropical North Pacific ODZ and amended with methane and nitrate. ODZs are a significant source of methane, and of nitrous oxide emissions with active microbial communities capable of reducing nitrate and oxidizing alkanes. Intriguingly, our enrichments consumed nitrate and produced nitrous oxide in the presence of methane. 16S rRNA sequences were most related to marine denitrifying Marinobacter (~89%) known to be capable of degradation of hydrocarbons and alkanes, and aerobic marine chemoheterotrophic Flavobacteriales. Catalyzed reporter depositional-fluorescent in-situ hybridization with a gammaproteobacterial probe (CARD-FISH, Gam42a, Marinobacter) indicate this uncultured Marinobacter dominated our cultures (>60%). Meta-omics surveys of the ETNP ODZ support Marinobacter being present and active players in both hydrocarbon oxidation and denitrification. Interestingly, we observed autofluorescence at 405-420 nm wavelength with >95% of the cells that hybridize with Gam42a (presumed Marinobacter). Autofluorescence has not been found in Marinobacter to date but has been found in other microbes at 420 nm associated with flavin pigments and cofactors. Work is ongoing for metagenomic sequencing, Marinobacter-specific FISH microscopy, and characterization of ideal artificial media for our enrichment cultures.

Session: Functional Omics 706
Presenter: Silver Sung-yun Hsiao
Title: Nitrogen stable isotope analysis in NanoSIMS 133
Authors Hsiao, Silver Sung-Yun, Institute of Astronomy and Astrophysic, Academia Sinica, Taiwan; Lee, Der-Chuen Lee, Institute of Earth Science, Academia Sinica, Taiwan; Shang, Hsien, Institute of Astronomy and Astrophysic, Academia Sinica, Taiwan

Nanometer-scale secondary ion mass spectrometry (NanoSIMS) has been used for frontier research in many fields including biology, medical science, material science, earth and planetary science, because it can analyze the lateral distributions of elements and their stable isotopes by down to 50 nm resolution. However, using weaker primary beam to reach better spatial resolution may lead to worse precision of isotopic ratios which is not good enough to differentiate natural variation. Applying 15N stable isotope tracer is one solution to make the d15N variation higher enough to be distinguished. Here we present three compromised and successfully published cases of nitrogen stable isotope analysis. The first one is probing extraterrestrial signal of organic nitrogen in meteorite. The second one is analyzing nitrogen fixation and methane assimilation activity of symbiotic type I methanotrophs in rice root. The last one shows how nitrogen isotopic signature from food is recorded in fish otolith. Welcome to discuss future collaboration with us.

Session: Functional Omics 707
Presenter: Madeleine A. Thompson
Title: Ammonia-oxidizing bacteria and archaea in the sediments and water column of brackish and salt marshes
Authors: Thompson, Madeleine, A.; Peng, Xuefeng, School of the Earth, Ocean, and Environment University of South Carolina

Marshes act as a transitional zone between uplands and coastal waters, providing a sink for anthropogenic contamination. As anthropogenic nitrogen loading increases, the production of greenhouse gasses and ozone-depleting agents, such as nitrous oxide, has increased through primarily biotic processes such as nitrification and denitrification. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are key drivers of biogeochemical processes in marsh sediments. Previous studies found that AOA were two orders of magnitude more abundant than AOB in salt marsh sediments. We used metagenomics to investigate the diversity and abundance of bacterial and archaeal ammonia monooxygenase (amoA) genes in the sediment and water column of a salt and a brackish marsh in South Carolina, USA. In salt marsh sediments, we did not detect any bacterial amoA gene, and nearly all archaeal amoA genes belong to the genus Nitrosopumilus. In brackish marsh sediments, the relative abundance of bacterial amoA genes was 43.5% higher than archaeal amoA genes, and most archaeal amoA genes were from uncultured Thaumarchaeota. The differential distribution of AOB and diversity of AOA suggest salinity played an important role in structuring ammonium-oxidizing communities. All bacterial amoA genes identified by metagenomics belong to the genus Nitrospira, suggesting a previously overlooked role of complete ammonia oxidation by this lineage in brackish marsh sediments. These findings shed new light on the ecology of ammonia-oxidizing communities in brackish and salt marsh sediments.

Early Career and Graduate student workshop at ICoN 8 on July 30, 2023

Schedule:
9.30 am: Coffee

10.00 am: Welcome

10.15 am: Round table about Job search for and working in industry with
    Lianna Poghosyan (Syngenta, Bracknell, UK)
    Shrameeta Shinde (Cemvita, Houston, TX, USA)
    Tomislav Ticak (Proctor and Gamble, Cincinnati, OH, USA)

11.00 am: Wei Qin (University of Oklahoma, Norman, OK, USA):
    Search for a Faculty position

11.30 am: Lisa Stein (University of Alberta, Edmonton, Canada)
    "The delicate Art of Balance"

12.00 pm: Lunch and campus walk

2.00 pm: Presentations by graduate students and Postdocs

Shuto Ikeda (Waseda University, Tokyo, Japan)
"Growth heterogeneity of divided cells affects the culturability of ammonia-oxidizing bacteria"

Jessica Bullington (Stanford University, Stanford, CA, USA)
"Distribution and diversity of nitrifying microorganisms within the subterranean estuary at Stinson beach, California"

Matthew Sima (Princeton University, Princeton, NJ, USA)
"The FEAMMOX process: Kinetics, batch incubations, and column studies in the presence of PFAS"

John Tracey (Columbia University, New York, NY, USA)
"All about nitrite: Exploring nitrite sources and sinks in the Eastern Tropical North Pacific Oxygen Minimum Zone."

Claire Elbon (Georgia Institute for Technology, Atlanta GA, USA)
"Autoflourescent *Marinobacter* dominate long term marine enrichments grown on methane and nitrate."

Around 3.30 pm: Wrap up and closing remarks

Organizers:
Samantha Fortin (Princeton University)
Madisen Kimbrel (Miami University)
Wouter Lenferink (Radboud University)
Weiyi Tang (Princeton University)
Annette Bollmann (Miami University)

ICoN8 Speaker Schedule

30 July	Sunday	**Opening Session**	
	1700 - 1800	Plenary	**Holger Daims** *An expanded picture of nitrification: comammox, alternative lifetyles, and microbial ineractions*
31 July	Monday	**Biochemistry/Bioenergetics**	
		Convenors	**Boran Kartal and Kyle Lancasater**
	830 - 915	Invited Speaker	**Thomas Barends** *Inside the Anammoxosome: Molecular Details of Anaerobic Ammonium Oxidation*
	915 - 920	Break	
	920 - 940		**Kyle Lancaster** *Maturation and Reactivity of Cytochrome P460*
	940 - 1000		**Gordon Williamson** *Site-seeing in ammonia monooxygenases*
	1000 - 1020		**Wei Qin** *Differential substrate affinity and catabolite repression enable preferential use of urea by ammonia-oxidizing bacteria*
	1020 - 1100	Break	
	1110 - 1130		**Paloma Garrido Amador** *Enrichment and characterization of nitric oxide-reducing microorganisms in a continuous bioreactor*
	1130 - 1150		**Beate Kraft** *Physiology of ammonia-oxidizing archaea under oxygen depletion*
	1150 - 1210		**Britt Abrahamson** *Hydroxylamine driven nitrate reduction: A novel anoxic pathway in the complete ammonia oxidizing bacteria Nitrospira inopinata*
	1210 - 1230		**Wouter Lenferink** *Alcaligenes faecalis accumulates hydroxylamine as a central intermediate during heterotrophic nitrification*
	1230 - 1400	Lunch	
31 July	Monday	**Biotechnology/Applications**	
		Convenors	**Ameet Pinto and Sukhwan Yoon**
	1400 - 1445	Invited Speaker	**Mari Winkler** *Unlocking the power of commamox and AOA to enhance engineered applications*
	1445 - 1450	Break	

	1450 - 1510		**Minsheng Jia** *Exploring the limits of novel thermophilic anammox*
	1510 - 1530		**Eva Spieck** *A novel Nitrospira lineage isolated from activated sludge using elevated temperatures*
	1530 - 1600	Break	
	1600 - 1620		**Jonathan Rodriguez** *Activity-based ammonia monooxygenase labeling is specific for ammonia oxidizing bacteria and reveals inner membrane localization of the enzyme*
	1620 - 1640		**Michelle M. McKnight** *Microbial community analysis reveals a dominance of either comammox Nitrospira or archaea as ammonia oxidizers in freshwater aquarium biofilters*
	1640 - 1700		**Freidman Lirone** *Characterizing nitrogen kinetics under hyper oxygen conditions in soil treatment using model simulation*
	1700 - 1720		**Akihiko Terada** *Phylogeny, abundance, and function of N2O-reducing bacteria in an anammox biofilm*
1 August	Tuesday	**Terrestrial/Soil/Agriculture**	
		Convenors	**Jeanette Norton and Graeme Nicol**
	830 - 915	Invited Speaker	**Gunter Subbarao** *Biological nitrification inhibition (BNI) - a nature-based solution for nitrogen problems in agriculture*
	915 - 920	Break	
	920 - 940		**Willm Martens-Habbena** *The role of soil nitrification in soil health, soil carbon retention, and greenhouse gas emissions*
	940 - 1000		**Soo Hyun Park** *Differential response of nitrate-reducing synthetic communities to varying carbon-to-nitrogen ratio*
	1000 - 1020		**Li Hanyan** *Divergent responses of soil nitrifying populations to long-term climate warming*
	1020 - 1050	Break	
	1050 - 1110		**Andrew Giguere** *Application of NH3 and pH optodes to visualize NH3 production and diffusion from fertilizer nitrogen and impacts on nitrification activity*

	1110 - 1130		**Paula Rojas-Pinzon** *Biological nitrification inhibitor efficacy depends on compound concentration, microbial community composition and is resilient to short-term soil pH changes*
	1130 - 1150		**Ida Peterse** *Elucidating acidic ammonia oxidation by "Candidatus Nitrosacidococcus tergens" sp. RJ19*
	1150 - 1210		**Xia Zhu-Barker** *Exploring feammox in cultivated wetland soils: A twelve-year incubation study and its implications*
	1210 - 1230		**Eunkyung Choi** *Oligotrophic ammonia oxidation in Nitrosocosmicus sp. R2*
2 August	Wednesday	**Global Nitrogen Cycling in a Warming World**	
		Convenors	**Bess Ward**
	900 - 945	Invited Speaker	**Emily Zakem** *Understanding and predicting nitrification at global scales*
	945 - 950	Break	
	950 - 1010		**Michael Beman** *Tropical cyclones accelerate the nitrogen cycle in the ocean's largest oxygen minimum zone*
	1010 - 1030		**Chris Francis** *Insights into massive seasonal ammonia-oxidizing archaea blooms in south San Francisco Bay*
	1030 - 1050		**Nicky Dotsios** *Microbial nitrogen cycling in a seasonally stratified marine lake*
	1020 - 1050	Break	
	1050 - 1110		**Claudia Frey** *Oxidative and reductive nitrous oxide production within the Amazon River plume*
	1110 - 1130		**Weiyi Tang** *Ammonia oxidation and anammox in the seasonally hypoxic Chesapeake Bay*
	1130 - 1150		**Xin Sun** *The explicit representation of diverse denitrifiers in a model resolves observed nitrogen dynamics in marine oxygen minimum zones*
	1150 - 1210		**Mark Trimmer** *Production of 15N2 gas from 15NH3 in oxic marine and freshwater sediments*

	1210 - 1230		**Mhlangabezi Mdutyana** *Nitrification in the Southern Ocean: controls, kinetics, and biogeochemical implications*
	1230 - 1400	Lunch	
2 August	Wednesday	**Symbiosis/Interactions**	
		Convenors	**Graeme Nicol**
	1400 - 1445	Invited Speaker	**Jana Milucka** *Diversity and distribution of denitrifying respiratory symbionts*
	1445 - 1450	Break	
	1450 - 1510		**Kateryna Zhalnina** *Chemoecology of Plant-Archaea Interactions via Linkages Between Metabolites, Genes, and Nutrient Cycling*
	1510 - 1530		**Lilly Blume** *Ammonia-oxidizing archaea associated with harmful algal blooms in the York River estuary*
	1530 - 1600	Break	
	1600 - 1620		**Katharina Kitzinger** *Metabolic interactions and genome evolution in marine AOA - sponge symbioses*
	1620 - 1640		**Man-Young Jung** *Competitive inhibition of ammonia-oxidizing microorganisms in co-culture systems at high and low ammonia concentrations*
	1640 - 1700		**Mara Walters** *Microplastics alter nitrification activity in the ammonia-oxidizing bacterium Nitrosospira sp. AV*
	1700 - 1720		**Maximilian Dreer** *Ammonia oxidizing archaea cultivated from human skin*
3 August	Thursday	**Functional Omics**	
		Convenors	**Lisa Stein and Sebastian Lucker**
	830 - 915	Invited Speaker	**Alyson Santoro** *Genomic and metabolomic approaches to understanding the ecology of marine nitrite oxidizer s*
	915 - 920	Break	
	920 - 940		**Xuefeng (Nick) Peng** *The ecology of active nitrifiers in the eastern tropical North Pacific oxygen minimum zone*

	940 - 1000		**Bongkeun Song** *Metaproteogenomic investigation of sedimentary nitrogen cycling communities responding to seawater intrusion in the Nakdong River, South Korea*
	1000 - 1020		**Juliet Johnston** *Dissolved Oxygen’s impact on nitrifying bacteria transcriptomic expression in a full-scale attached growth wastewater treatment system*
	1020 - 1050	Break	
	1050 - 1110		**Graeme Nicol** *Identifying novel virus families infecting nitrifying archaea and bacteria in soil*
	1110 - 1130		**Bradley Tolar** *Environmental changes impact growth, transcription, and protein production of a low-salinity ammonia-oxidizing archaeon*
	1130 - 1150		**Logan Hodgskiss** *Filling gaps in the metabolism of ammonia oxidizing archaea using comparative and functional genomics*
	1150 - 1210		**Petra Pjevac** *Distinct physiological functions of NXR homologues in Nitrospira moscoviensis*
	1210 - 1230		**Rui Zhao** *A novel bacterial phylum involved in nitrite consumption in marine sediments*
	1230 - 1400	Lunch	
3 August	Thursday	**Closing Session**	
	1400 - 1600	Plenary	**Sukhwan Yoon** *Quest for deciphering environmental N2O sinks - ecophysiology of high-affinity N2O reduction* **Boran Kartal** *Welcome to ICoN9*

International Conference on Nitrification and Related Processes - Sunday, July 30 - Thursday, August 3, 2023

Session name	poster nu	Presenter First name	Last name	Title
Early Career Workshop	101	Ayano	Kaneko	Isolation and physiological characterization of a novel nitrite-oxidizing bacterium, Nitrobacter sp. A67 which accommodates to moderately acidic pH from agricultural soil
	102	Parker	Lawrence	Seasonal dynamics of marine and estuarine Thaumarchaeota in coastal north carolina
	103	Marcel	Malinowski	Potential novel nitrifying bacteria from a freshwater recirculating aquaculture system (ras) and their ecological niche formation
	104	Thomas	Pribasnig	The biofilm lifestyle of ammonia-oxidizing archaea: an understudied mode of growth and activity
	105	Yuki	Shiraishi	Temperature dependence in nitrite oxidation and gene expression by a Nitrobacter sp.
	106	Alexander	Umbach	Succession of freshwater aquarium nitrifying communities in response to fish load
Biochemistry/Bioenergetics	201	Justine	Albers	Adapting thermal shift assays to functionally characterize nitrifier substrate binding proteins
	202	Jemma	Fadum	Using resource competition theory to explore the tradeoff between DNRA and denitrification in aquatic ecosystems
	203	Jessica	Holechek	In vitro assembly of ammonia monooxygenase into nanodiscs
	204	Shuto	Ikeda	Growth heterogeneity of divided cells affects the culturability of ammonia-oxidizing bacteria
	205	Megumi	Kuroiwa	Quantitative evaluation of nitrous oxide sink capacity of denitrifying bactera by a newly developed dual-tracer method
	206	Donald	Martocello	Physiological insights on bacterial and archaeal ammonia oxidation from variable stable isotope dynamics in response to metal limitation
	207	Benjamin	Shapero	Characterization of structural importance of nirk sequence diversity from AOA
	208	Bongkeun	Song	Abiotic oxygen production from nitrite decomposition in acidic conditions
	209	Hidetoshi	Urakawa	Nitrosomonas supralitoralis sp. Nov., a betaproteobacterial ammonia-oxidizing bacterium isolated from beach sand in a supralittoral zone, Washington, USA
	210	Bram	Vekeman	Versatile anaerobic ammonium-oxidizing bacteria can use alternative carbon and nitrogen sources for growth and energy conservation
	211	Rob	Voland	Structural and biochemical studies of novel enzymes implicated in nitrogen cycling
	212	Wei Qin and Britt Abrahan		Ammonia-oxidizing microorganisms employ distinct strategies coordinating ammonia and urea metabolism
Biotechnology/Applications	301	Eleftheria	Bachtsevani	In vitro assessment of pesticide toxicity on soil nitrifying microorganisms: a novel tool for ecotoxicity risk assessment
	302	Jiyun	Li	Comammox nitrospira dominated in a low dissolved oxygen nitrifying reactor
	303	Mohammad	Mozaffari	Influence of media size and plant species on nitrification in unsaturated vertical flow wetlands
	304	Satoshi	Okabe	Oxygen tolerance and detoxification mechanisms of anammox bacteria
	305	Yung-Hsein	Shao	Comammox Nitrospira Cooperate with Anammox Bacteria in a Partial Nitritation–Anammox Bioreactor Treating Low-strength Ammonium Wastewater at High Loadings
	306	Christiana	Abiola	Differential growth of ammonia-oxidizing archaea on floating filter
	307	Han-Yin	Chuang	Effects of glutamate on population dynamics of anammox consortia associated with salinity changes
	308	Akito	Oishi	Genomic and physiological insights into N2O emissions by cluster 7 ammonia-oxidizing bacteria isolated from partial nitrifying bioreactors
	309	Mamoru	Oshiki	Remineralization of organic nitrogen compounds into ammonium in marine anammox bacterial culture
	310	Lisa	Stein	Inoculum and ph effects on ammonium removal and microbial community dynamics in aquaponics systems
	311	David	Vuono	Continuous Measurements of NOB Growth through the application of photoconduction in a Microbial Growth Intervalometer (MAGI).
	312	Shih-Wei	Yeh	Membrane oxygenation-driven anammox biofilm reactor treating low-strength ammonium wastewater
	313	Mensheng	Jia	Does AOA-driven thermophilic nitritation have lower N2O emissions?
Terrestrial/Soil/Agriculture	401	Shannon	Brown	Linkage of nitrification and denitrification and its impact on carbon cycling in tropical agricultural soils
	402	Hirotsugu	Fujitani	Physiology and genomics of a soil nitrite-oxidizing bacterium, Nitrobacter sp. Cn101 with high affinity to nitrite
	403	Jeanette	Norton	Nitrification communities in maize agroecosystems
	404	Matthew	Sima	The feammox process: kinetics, batch incubations, and column studies in the presence of PFAS
	405	Chiara	Smorada	Assessing the impact of pfas on nitrifying and denitrifying microbial communities in wetland soils
	406	Daan	Speth	Genetic potential for nitrogen cycling metabolism in Denmark
	407	Edi	Wipf	Deciphering nitrogen flow networks in grassland biomes: how rhizosphere metabolomes drive nitrifier community dynamics in a Mediterranean climate zone
	408	Akari	Kimura	Diversity of soil microbial community is linked to the stability of ammonia-oxidizing bacteria and archaea community and nitrification rates in a volcanic ash soil
	409	Jun	Zhao	Nutrient acquisition strategies drive coexistence patterns among the dominant lineages of soil ammonia oxidizing archaea
	410	Sakiko	Okumoto	Pathways to increase biological nitrification inhibition
	411	Nithya	Rajan	Field-scale Nitrous Oxide Flux from Sorghum in the U.S. Great Plains using Eddy Covariance
	412	Karpouzas	Dimitrios	Synthetic microbial communities of nitrifiers: a tool for ecologically relevant assessment of pesticides toxicity on soil microbes
Global N Cycling in a Warmin	501	Jade	Bosviel	Spatiotemporal dynamics of nitrososphaera-driven nitrification and CO2 fixation in a perialpine lake
	502	Pearse	Buchanan	Dynamic oxygen intrusions sustain aerobic nitrite oxidation in anoxic marine zones
	503	Elisa	Hernández-M	The role of nitrous oxide (N2O) in the metabolism of ammonia-oxidizing archaea (AOA) under oxygen-depletion
	504	Kanae	Kobayashi	Microbial nitrogen cycling in the deep-sea sediments: combined molecular and stable isotopic analyses to determine the nitrogen flux of nitrification and anammox

	505	Hou	Lei	Ocean warming enhances iron use efficiencies of marine ammonia-oxidizing archaea
	506	Zicheng	Su	Comammox Nitrospira dominated as scavenger for urea as nitrogen source
	507	Xianhui	Wan	Pathways of N2O production by marine ammonia-oxidizing archaea determined from dual isotope labeling
	508	Dongyoung	Back	The effects of dam gate opening on nitrogen cycling in Nakdong River estuary with an idealized biogeochemical model
	509	Carolyn	Buchwald	Using rate incubations and the stable isotopic composition of nitrate to determine the amount of nitrification, regeneration, and new production during a bloom in the Labrador Sea
	510	Jessica	Bullington	Distribution and diversity of nitrifying microorganisms within the subterranean estuary at Stinson Beach, California
	511	Paula	Garcia Otero	Ecology and physiology of ammonia-oxidizing archaea under sulfidic conditions
	512	Naomi	Intrator	Nitrite reductase gene (nirk) phylogeny and biogeography
	513	Clarissa	Karthauser	Investigating nitrogen cycling in marine particles while keeping them afloat
	514	Akiko	Makabe	Nitrification rates in the eastern Indian Ocean
	515	John	Tracey	All about nitrite: Exploring nitrite sources and sinks in the Eastern Tropical North Pacific Oxygen Minimum Zone
	516	Zihiuzhong	Ye	Anthropogenic impact on nitrifying and denitrifying microbial communities in coastal zone
	517	Chisato	Yoshikawa	Development of a marine ecosystem model for impact assessment of acidification on nitrification
Symbiosis/Interactions	601	Sarah	Al-Ajeel	Competition versus cooperation: Ecological strategies of nitrifiers
	602	Nicole	Geerlings	From the single cell to the community: metabolic heterogeneity in comammox Nitrospira
	603	Grace	Kennedy	Friends with benefits? Exploration of symbiotic ammonia-oxidizing archaea within ascidian tunic microbiomes
	604	Madisen	Kimbrel	Oxidative stress alters the heterotrophic community structure of ammonia-oxidizing enrichment cultures.
	605	Sabita	Ghimire	Competition for ammonium between a complete ammonia oxidizer and an ammonia-oxidizing archaea from a freshwater lake in Ohio
Functional Omics	701	Samantha	Fortin	Novel nitrite oxidizing bacteria perform nitrite oxidation in oxygen minimum zones
	702	Birch	Lazo-Murphy	Transcriptomic analysis of the nitrous oxide-producing salt marsh sediment fungi, Purpureocillium lilacinum
	703	Christopher	Sedlacek	The role, or lack thereof, of sNOR during denitrification by ammonia-oxidizing bacteria
	704	Olabisi	Adebisi	The ecophysiological relevance of low salinity in niche differentiation of ammonia oxidizing microorganisms
	705	Claire	Elbon	Autoflourescent Marinobacter dominate long term marine enrichments grown on methane and nitrate
	706	Siver	Sung-Yun Hsi	Nitrogen stable isotope analysis in NanoSIMS 133
	707	Madeline	Thompson	Ammonia-oxidizing bacteria and archaea in the sediments and water column of brackish and salt marshes