N_2O Formation and Organic Nitrogen Utilization in Wetland Microbial Communities

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Project Goals: Wetlands capture and release large amounts of greenhouse gases (CO_2, CH_4 and N_2O) and it is of prime importance to predict their response to climate change induced stressors such as drought and sea level rise associated saltwater intrusion. This project aims to link wetland microbial activities to ecosystem-scale processes by developing a reproducible experimental model for lacustrine and estuarine wetland ecosystems to quantify responses to controlled manipulations representing climate impacts. Hydrogel beads, controllable in size, with entrapped wetland microbes and soil and plant-like carbon, act as models for sediment aggregates. Bioreactors with real-time gas and liquid metabolite flux monitoring, integrated multi-omics analyses, and stable isotope tracing will be conceptually incorporated into mathematical models to predict how climate change stressors impact C and N fluxes across different wetland spatial and temporal scales.

Overview: Nitrous oxide (N_2O), a potent GHG (265 times stronger than CO_2), is released in wetlands by heterotrophic denitrification as well as ammonia-oxidizing organisms (AOO). The three types of AOO are 1) ammonia oxidizing bacteria (AOB) which are prevalent in ammonium laden soil and emit the largest flux of N_2O 2) ammonia oxidizing archaea (AOA), which are ubiquitous in oligotrophic environments and 3) comammox bacteria, which were recently discovered and have redefined our understanding of nitrification by performing both ammonia oxidation and nitrite oxidation. Due to their novelty, the contribution of N_2O emissions from comammox bacteria remains under explored. Previous AOO investigations have focused on ammonia as the primary N substrate, despite organic nitrogen compounds being more prominent in wetland ecosystems. To better understand N_2O emissions in wetlands from AOOs as well as their organic nitrogen utilization patterns we **a)** explored the role of aerobic and anaerobic comammox metabolisms on N_2O emissions and **b)** studied the physiology and regulations controlling the use of alternative nitrogen sources (inorganic and organic) by different AOOs. In addition, we also studied the effect of drought events on N_2O emissions originating from nitrification and denitrification events (**c**).

(a) N_2O emissions from comammox bacteria under aerobic and anaerobic conditions

We confirmed aerobic N_2O production by comammox and could reproduce the recently published stoichiometry of N_2O emitted per ammonium oxidized (*1*). In addition, we studied the use of alternative electron donors and acceptors by comammox. We measured N_2O production during anaerobic comammox incubations and are currently investigating if it is the result of an abiotic conversion. These observations suggest that nitrifier induced N_2O production could be induced by comammox under physiologically relevant conditions. The results also indicate the role of comammox within environmental systems might be more complicated than previously thought.

(b) Physiological Studies of the use of alternative nitrogen sources in AOO

We characterized the anabolic incorporation and metabolic respiration of different inorganic and organic nitrogen sources in different AOO isolates, to determine N resource utilization and regulation among phylogenetically distinct AOOs. Isolates were fed organic and/or inorganic nitrogen substrates in batch

incubations. The organic nitrogen, ammonia, and nitrite concentrations were measured over time to determine substrate utilization. Stable isotope (^{15}N and ^{13}C) labeled organic nitrogen, ammonia, and bicarbonate were used to determine cellular respiration products and intermediates via Isotope-Ratio Mass Spectrometry (IRMS) and biomass incorporation using Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS). Significant differences in N drawdown patterns were observed among different AOO isolates. For example, organic N was consumed rapidly in β-AOB, being drawdown at higher rates than inorganic N. We confirmed the selective incorporation of organic-N over inorganic-N by β-AOB with NanoSIMS. In contrast to β-AOB, soil AOA preferentially used inorganic-N over organic-N. These observations suggest that phylogenetically distinct AOO have different mechanisms for nitrogen transport and utilization and challenge the assumption that ammonia N is always the primary substrate for ammonia oxidizers growth. Stable isotope tracking of $^{15}NO_2^-$ production is in progress to determine the dynamics in different N-substrates utilization for each AOO. Results from microrespirometry showed the differences in affinities amongst different AOO and nitrogen sources. RNA-seq and proteomics analyses are underway to infer regulatory mechanisms, which will lead to a better understanding of nitrogen partitioning and ecological niches across different nitrifiers.

(c) N_2O emissions at varying water table submergence

To understand the competition among nitrifiers and their interplay with denitrifiers in terrestrial wetlands (Lake Washington, WA) we conducted laboratory soil column experiments. This experimental model system was exposed to water table changes to understand the effect of climate change induced drought or flooding/inundation on globally significant microbially-mediated lacustrine wetland nitrogen cycling. GHG flux measurements indicate that the wetland soil columns were an N_2O-neutral system when submerged, but shifts to become an N_2O source (0.5 nmol s^{-1} m^{-2}) when the water table decreases. As a next step, we will encapsulate the wetland community in hydogels to generate a simplified microbial system. These results will be used to develop predictive models that link microbial physiology of terrestrial systems to overall ecosystem processes (e.g., nitrate leaching and N_2O emissions). The model will be used to simulate how climate change induced environmental changes such as different redox gradients, e.g. the change in oxygen concentration on a micrometer scale within individual hydrogels, impact GHG emissions, microbial abundance, and activities.

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References

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