## Explaining and Predicting Spatial Variation in Wetland N2O Emissions Through Microbial Functional Gene Associations

### Overview

*This section should provide a brief background description of the current knowledge gaps, limitations of the current state-of-the-art, and potential technological societal impacts that are the motivation for the proposed effort. This section should also describe the merits of the approach being used and how the team is uniquely positioned to address these challenges, recent technological/social advances that make the effort timely, and any other broader impacts that may benefit from these advances.*

Wetlands are a widely recognized organic carbon (C) sink, however recent attention has shifted toward the increases in atmospheric concentration of greenhouse gases like nitrous oxide (N2) leading toward the steady warming of the earth’s climate, and the potential contribution from wetlands. Since 1970, the total area of natural wetlands has declined by approximately 0.52% annually due to intensive land-use changes, including drainage and conversion to agriculture or urban areas, with significant consequences for N2O emissions (Robertson et al. 2025). While environmental drivers such as temperature, soil chemistry, and land-use have been well studied, the functional role of microbial communities in regulating global N2O fluxes, and how these contributions vary across space, remain poorly understood.

Recent technological advances, including high-throughput metagenomics and quantitative polymerase chain reaction (qPCR) for functional genes have enabled scientists like Brahram et al. to quantify microbial drivers of N2O emissions across wetlands at a global-scale in their study *Structure and Function of the Soil Microbiome Underlying N₂O Emissions from Global Wetlands* (2022). Using statistical models and machine learning approaches, they link microbial functional genes to observed N*2*O fluxes across a range of environmental variables such as climate, soil chemistry, and land-use, highlighting the potential use of ammonia-oxidizing archaea (AOA) and diversity of N-cycle genes (*amoA, nosZ*) in predicting N2O emissions. Despite the comprehensive scope of Bahram et al.’s study, questions pertaining to the unique contribution of microbial functional genes to spatial variation in N₂O emissions and the degree of functional complementarity (*i.e*. co-existence of multiple species resulting in a larger overall functional performance than that of individual performance) between nitrifiers and denitrifiers across environmental gradients remain unanswered.

*(**Blüthgen & Klein 2011)*

The global-scale dataset from by Bahram et al (2022), which includes absolute quantification of N-cycle functional gene abundances and in-situ N2O fluxes across associated environmental covariates from 645 wetland sites worldwide provides a foundation for addressing these gaps. This project aims to build on these findings by leveraging spatial analytic techniques to assess the relative influence of microbial functional genes on N₂O emissions beyond environmental factors, and to explore how the functional complementarity between nitrifiers and denitrifiers drives spatial variation in N2O emissions across wetlands.

### Goals and Objectives

*This section should briefly describe the overall goal(s) of the project and the subsequent objectives that will enable them to be achieved. Goals and objectives should be directly connectable to the tasks outlined in the research and collaboration plan.*

This project aims to further assess the role of microbial functional genes in driving spatial variation of N2O emissions across wetlands, beyond the influence of environmental factors such as climate, soil, and land-use. By integrating microbial and environmental data within a spatial framework, this study seeks to better understand how microbial composition and interactions shape patterns of N2O fluxes across landscapes. Our two specific and measurable objectives include:

1. **Quantify the contribution of** **microbial functional genes**—including archaeal *amoA*, bacterial *amoA*, and *nosZ*, and overall N-cycle gene diversity—**to spatial variation in N2O emissions**, assessing the **explanatory power** provided by microbial factors beyond environmental variables.
2. **Investigate the functional complementarity** between nitrifiers (*amoA*) and denitrifiers (*nosZ*) across space, examining how the **balance** between these microbial groups relate to spatial patterns of N2O emissions.

### Research Plan (task-based with timeline)

*Outline the proposed research plan into logical tasks with an emphasis in each task, breaking down the work into logical tasks. Each paragraph that follows could be for a separate task as shown below. The focus should be on describing the tasks, the reasons for completing the task, how the tasks will be completed, how and which of the learnings from course modules would be leveraged in each task, and the likely outcomes of the proposed work. Take care to define a scope of work appropriate for the amount of effort required for coursework equivalent credit hours.*

***Task 1:* Quantify the added explanatory power of microbial genes**

***Task 2:***

Linkages between tasks are encouraged.

### Evaluation Metrics

*Briefly describe the quantitative and/or qualitative metrics that will be used to determine the progress and success of each task, progress towards objectives, and the overall project goal. These metrics will be used to determine how your project outputs will be evaluated for course credit.*

### Anticipated Outcomes & Impacts

*Describe anticipated outcomes and experiences relevant to the topical area. These can include, but are not limited to, technical deliverables, science/engineering advances, education impacts, or policy changes.*

### Follow-up Funding/Follow-up Research/Growth Plan

*Expectations for this effort is that the proposer will use the initial works that was for coursework to develop their own research initiative that can become self-sustaining and has the potential to grow into a larger research initiative after course completion. The proposer should briefly lay out a plan for achieving self-driven subsequent research, and potential sources of funding and the timing for their pursuit.*

## References

Bahram, M., Espenberg, M., Pärn, J. *et al.* 2022. Structure and function of the soil microbiome

underlying N2O emissions from global wetlands. *Nat Commun* **13**, 1430 <https://doi.org/10.1038/s41467-022-29161-3>

Robertson, H., et al. 2025. Global Wetland Outlook 2025: Valuing, conserving, restoring

and financing wetlands. Gland, Switzerland: Secretariat of the Convention on Wetlands. DOI: 10.69556/GWO-2025-eng.

1. To what extent do microbial functional genes explain spatial variation in N₂O emissions beyond climate, soil, and land-use factors?

* H₀: Microbial functional genes explain no additional spatial variance in N₂O flux once climate, soil, and land use are considered.
* H₁: Microbial functional genes uniquely explain a significant portion of N₂O spatial variance.

1. Cross-scale variance partitioning: how much of spatial variation in N₂O is explained by climate, soil, land-use, vs microbial genes?

Hypothesis: A large fraction of spatial variance in N₂O is explained by microbial functional gene metrics beyond climate/soil/land-use.  
Variables: sets of predictors grouped: climate (temp, precipitation), soil (pH, C/N, moisture, NO₃⁻), land use, microbial (amoA, nir, nosZ, gene diversity).

1. are high amoA sites associate with high nosZ sites? (neighborhood effects)

Hypothesis: High archaeal amoA sites are spatially followed by neighboring sites with higher nosZ (N₂O sink), indicating spatial complementarity of nitrification and consumption.

1. Does the balance between nitrifiers and denitrifiers, measured as the ratio of archaeal amoA to nosZ gene abundance, improve prediction of N₂O emissions across global wetland soils when combined with climate, soil, and land-use factors?
2. *Does the predictive power of the amoA:nosZ ratio vary across spatial or environmental gradients (e.g., latitude, temperature, or land-use intensity)?*

* **H₀ (Null):** The relationship between the amoA:nosZ ratio and N₂O emissions is consistent across spatial and environmental contexts.
* **H₁ (Alternative):** The strength and direction of the amoA:nosZ–N₂O relationship vary spatially, with stronger effects in warmer, drained, or intensively used wetlands.

**Task 1 — Quantify the added explanatory power of microbial genes**

**Objective:** Assess how much spatial variation in N₂O emissions is explained by microbial functional genes (amoA, nir, nosZ, and gene diversity) beyond climate, soil, and land-use factors.

* **Hypothesis:** Microbial functional genes uniquely explain a significant portion of N₂O spatial variance not captured by environmental factors alone.
* **Approach:** Variance partitioning (e.g., hierarchical regression, spatial R² decomposition, or Moran’s Eigenvector Maps) to quantify unique vs shared contributions.