# The Importance of Microbial Community Structure in Determining Methane Flux Variability of Resorted and Natural Freshwater Wetlands

## Introduction

Wetlands have the ability to emit, or flux, large amounts of methane, a potent greenhouse gas[1]. Wetland methane emissions have thus become an increasingly important area of research over the past few decades, as atmospheric methane concentrations continue to rise [2]. Wetlands also have the ability to sequester large amounts of carbon, holding almost 20% of the world's soil carbon pool, due to slowed decomposition rates in saturated soils. Understanding both when and why wetlands become either sources or sinks of atmospheric carbon can thus have important implications in the context of climate change[3,4].

Freshwater wetlands especially have the capacity to emit large amounts of methane to the atmosphere but observed methane emissions from these wetlands vary widely [5–7]. This variability emphasizes the importance of processes occurring within the wetland. For instance, microbial methane production, or methanogenesis, which is responsible for methane production in wetlands, is driven by numerous environmental factors. These factors include hydrology, vegetation, temperature, pH, and carbon availability, all of which can vary widely between and within wetlands[6]. At the same time, other factors and processes constrain the portion of methane that is produced during methanogenesis that eventually reaches the atmosphere [5,8,9]. One of these constraints is methane oxidation through microbial methanotrophy. Unlike methanogenesis, which can only be performed in anoxic, saturated soils, methane oxidation can be performed in both saturated and unsaturated, or anoxic and oxic, soils. These two processes are called anaerobic and aerobic methane oxidation, with aerobic methane oxidation being more energetically favorable and thus more prevalent [10,11]. Methane oxidation dynamics, both aerobic and anaerobic, in wetlands are thus essential for determining methane flux variability.

Restored wetlands also show high variability in methane emissions, with some restored sites showing relatively elevated flux rates[5,6]. The reason for this is not completely understood, partially because it is hard to know what factors may be driving methane biogeochemistry in a particular system at any given time. For instance, some restoration sites experience altered soil structure, hydrology, vegetation, as well as seasonality [12,13].

At the same time, even though it is environmental factors that broadly drive microbial methane production and oxidation, it is not well known how important these factors are for determining the microbial community structure itself. This is important as recent research suggests that understanding microbial community structure is more important for understanding subsequent ecosystem function, such as methane production or oxidation, than previously thought. In short, in order to better understand the variability of methane flux in wetlands, it is important to know how microbial community structure mediates the connection between environmental drivers, like hydrology, and ecosystem processes, like methane flux [14,15].

## Research Objectives and Hypothesis

The **objective** of this work is to understand the variability in methane flux rates across a hydrological gradient in restored and natural freshwater wetlands by drawing the connection between environmental drivers, microbial community, and ecosystem function. To address this, we will **1.)** identify potential environmental drivers of differences in microbial community structure and subsequent function by assessing soil structure, edaphic variables, and annual hydrology. **2.)** assess the soil microbial community structure by performing sequencing of the

universal marker gene 16s rRNA and **3.)** determine the associated ecosystem function by measuring methane production and oxidation potential rates.

We **hypothesize** that **1.)** restoration sites will show altered environmental drivers in relation to natural sites, specifically an annual water table above the soil surface for a longer portion of the year, more soil compaction, and less iron availability. These drivers will correlate with a loss in overall microbial community diversity, and a shift in relative community abundance, including more methanogens (taxa: *Methanosaetacea, Methanosaricihae,* and *Methanocellales*), and fewer methanotrophs (group I and II aerobic methanotrophs and ANME-1 and ANME -2 anaerobic methanotrophs). Subsequently, we expected to see higher relative potential rates of methane production, and lower potential rates of methane oxidation, leading to higher estimated methane flux rates across restored sites. We expect **2.)** that all six wetlands will show variability across the hydrologic gradient, with the open water centers having the highest relative abundance of methanogens and higher rates of methane production. We expect the transition zone to have the highest methane oxidation, both aerobic and anaerobic, potential rates, and thus the highest relative abundance of methanotrophs. We expect this difference to be primarily driven by annual water table in each zone.

## Methods

### *Soil collection and Analysis of Environmental Factors*

Six Delmarva Bay wetlands located on the Delmarva Peninsula in Maryland, USA, will be used in this experiment, three natural and three restored. These wetlands are characterized by their mineral soils and experience seasonal hydrology, with the water table rising in the late spring and remaining high until early autumn when the water table drops. Soil will be collected from three zones along a hydrologic gradient in all six wetlands: the open water center, which experiences the longest saturation duration, the transition zone, which experiences the most variable saturation, and the forested edge, which experiences the shortest saturation duration. Soil cores will be taken to 30cm depth, bulked, and transported at 4°C to the lab for incubation, microbial community sequencing, and soil analysis.

Soil temperature, pH, and resistance to penetration, a measure of compaction, will be measured in the field at the time of sampling. Ferric and Ferrous iron concentrations will be calculated in the lab using a protocol adapted from Hach Method 8146 [19]. Microbial biomass carbon, which will inform the relative size of our microbial communities, will be measured using the chloroform fumigation method and analyzed using a Shimadzu total organic carbon analyzer[20]. Soil organic matter will be calculated as loss on ignition and soil water content will also be obtained by oven drying to constant weight. Soil texture analysis will be done using the hydrometer method and bulk density will be calculated as soil weight by volume. All proposed sites are instrumented with continuously logging water table monitors deployed in 2-meter wells. These instruments are positioned to capture the hydrologic variability within sites.

### *Incubations*

Mesocosm incubations for aerobic methane oxidation, anaerobic methane oxidation, and methane production potential rates will be performed in triplicate for each hydrologic zone (n=3) of each wetland (n=6). These incubations will be established with 20g soil aliquots in 50ml glass vials, that will then be capped to maintain gas headspace. Anaerobic methane oxidation and methane production incubations will be performed at 100% water holding capacity with an anoxic, $N_2$, headspace. Anaerobic methane oxidation incubations will have methane added to the

headspace and consumption of that methane will be monitored over time to calculate rate of oxidation. These incubations will have 2-bromoethanesulfonate (BES) added as an inhibitor of methane production, as to isolate methane oxidation. Aerobic methane oxidation incubations will be performed using the same protocol but with an oxic, $N_2$+ $O_2$, headspace and kept at a water holding capacity equivalent to the lowest condition observed *in situ*. Mesocosm headspace will be sampled daily for six days and samples will be analyzed using a Gas Chromatograph (SRI 8610C) for methane and carbon dioxide concentration. Rate potentials will be calculated as either the moles of methane produced, or consumed, per gram of dry soil, per sampling period. The production and oxidation potential rates in each wetland zone will be compared to estimate the percent of methane produced that is oxidized, giving a rough estimate of atmospheric flux or methane emission. This estimated flux will be contextualized by comparison with flux tower eddy covariance data collected at one of the natural and one of the restored sites, as well as previous methane chamber data collected by members of the lab.

***Sequencing***

DNA extraction and isolation will be performed following QIAGENS's Powersoil DNA extraction kit protocol and bacterial and archaeal 16s rRNA will be quantified using primers chosen according to the earth microbiome guidelines[21]. Sequencing will be performed using the Illumina MiSeq platform and v3 reagents targeting the 16s rRNA gene (515/806 bp). MiSeq outputs will be analyzed using R Statistical Programming and the dada2 package (version 1.6). The dada2 package will be used to clean, rarify, and check the quality of the sequence data. We will then make taxonomic assignments by comparing sequences to either the SILVA (SILVA v128, arb-silva.de) or RDP (v11.5, rdp.cme.msu.edu) databases. The final table of operational taxonomic units (OTUs) will be analyzed using the R phyloseq Package (v1.2) and used to determine beta and alpha diversity within, and between, our treatment groups.

**Broader Implications and Benefit to Coastal Wetlands**

The Delmarva Peninsula was once covered in thousands of Delmarva Bay wetlands, but over the last century many of these have been converted for agriculture. More recently, protection has been politically controversial due to the wetland's status as geographically isolated, and thus federally unprotected. Despite this research continues to show these wetland's regional hydrologic connectivity and importance in maintaining water quality and nutrient cycling, as well as biodiversity, in the broader region, including the Chesapeake Bay[16–18]. We hope that by showing the dynamic biogeochemistry of intact wetlands our work will help demonstrate why keeping these wetlands intact and functional is essential for providing key ecosystem service across the region, making a case for increased conservation. This case for conservation is essential as wetlands like these are incredibly vulnerable on the federal level. In addition, Previous site restoration and assessment has occurred in these sites as part of the U.S. Department of Agriculture's Wetlands Reserve Program and subsequent Conservation Effects Assessment Project. Our work thus builds upon a larger effort to understand the efficacy of restoration and conservation in these wetlands [13]. Our broader goal is to help inform the understanding of wetland ecosystem processes, so as to improve future restoration and conservation practices.

**Work Completed**

Wetland site selection for this project has been completed, and access has been granted by landowners. Our first soil sampling campaign is scheduled for Spring 2020. Soil incubations using the same mesocosm design have been performed to confirm both the presence of methane oxidation and production in these soils, as well as to validate the methodology proposed here, especially in regard to the ability of BES to suppress methanogenesis. In pre-incubations, soil from the open water center and forested edge of one natural wetland showed much higher methane oxidation potentials than soil from the same zones in a restored site. The restored site zones also showed higher overall methane production potential rates. This was validated by comparison with data of methane production rates calculated from both a methane chamber study and from flux tower eddy covariance data in the same two sites, which is part of an in-prep manuscript by members of the Palmer Lab at the University of Maryland.

**Fund Allocation**

The funding from this scholarship will be split equally between three portions of the project. The first part of the budget will be used for field costs associated with sampling supplies, instrument upkeep, and transportation. This includes maintenance of the well loggers and travel to sites. The second part of the budget will go towards lab analysis and instrument use, specifically to purchase gasses and reagents associated with the proposed incubations and protocols. The funds will also go towards the use of the Shimadzu total organic carbon analyzer, an integral part of understanding the associated biomass of the microbial community. The final part of the budget will be used for costs associated with microbial DNA sequencing. Sequencing of DNA is a multistep process requiring the use of expensive instrumentation, reagents, and specialized lab materials.

**Science Communication**

A poster presentation on the existing preliminary data from the one restored and natural site has been accepted for the American Society of Microbiology Washington D.C. Branch meeting in February 2020. This meeting is focused on the use of microbes in environmental research. A presentation focused on a larger portion of the data, has been submitted for the Society of Wetland Scientists' annual meeting in June 2020, which is focused on wetland restoration, reclamation, and rewilding. The goal is to present the complete findings at two more conferences once complete, including possibly the American Geophysical Union's annual meeting or again the Society of Wetland Scientists' annual meeting. The intent is to publish the eventual manuscript in a journal focused on applied science, like Applied and Environmental Microbiology, or to reach a broader audience, Restoration Ecology.

I am currently a global STEWARDS NSF training fellow at the University of Maryland. This fellowship comes with $1,500 in travel funds for attending and presenting at conferences. The fellowship also includes participation in professional development, community outreach, and mentoring. This means that through the fellowship program I will not only have many opportunities to share my work but will be trained to become a better communicator and scientist.

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