THESIS

DECIPHERING THE BIOLOGICAL DETERMINANTS ON METHANE CYCLING FROM GULF COAST WETLANDS

Submitted by

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ABSTRACT

DECIPHERING THE BIOLOGICAL DETERMINANTS ON METHANE CYCLING FROM GULF COAST WETLANDS

In Chapter 1, I introduce the importance of coastal wetlands for ecosystem services, including carbon storage, physical barrier for natural disasters, and habitat for diverse fauna and flora. Sea level rise is one of the main environmental risks affecting coastal wetlands, because of their geographic position. The effects of saltwater intrusion into freshwater wetlands can change established environmental conditions and vegetation coverage, which affects the functionality of various ecosystem functions they provide. These changes can also affect the methane emissions from coastal wetlands, which are major sources of this potent greenhouse gas. In this chapter, I evaluate the current knowledge of microbial methane production and consumption, including aspects of the ecophysiological adaptation to salinity, and the changes in the microbial ecological interactions modulated by increased salinity from marine water intrusion.

In Chapter 2, I conducted a study to characterize the microbial communities and geochemistry of soil and water compartments in three coastal wetlands following a salinity gradient from Barataria Bay, Louisiana. To investigate the methane cycling microbial communities and their distribution on a freshwater flotant, Jean Lafitte swamp, and saltwater marsh wetlands, I collected soil and water samples under different vegetation coverage from each wetland. I analyzed the 16S rRNA gene sequencing and paired this data with *in situ* methane fluxes and porewater concentrations. I also analyzed the geochemistry of the soil samples including profiling the anions, cations, pH, and redox conditions of soil and water

samples across wetlands. The analysis showed that the diversity of methane cycling microbial communities decreased with increased salinity. Although the distribution and relative abundance of methanogen functional types was not impacted, with hydrogenotrophic methanogens being the most abundant across all wetlands. Looking at the methanotroph abundance and taxonomy in soil and water samples, I observed that swamp and saltwater wetlands share more methanotroph members in the water column, while the soils had more site-specific similarities. My research findings contribute to the understanding of methane cycling microbial tolerance to saltwater and may be used in future works to create more robust methane prediction models.

In Chapter 3, I summarize the key aspects of microbial methane cycling in coastal wetlands and offer future directions for pairing geochemical and microbial data, including using an 'omics' approach and expanding investigation to more wetlands. We discuss the valuable findings these tools can give, contributing to a more accurate prediction of the metabolisms behind the ecophysiology and ecology of methane fluxes in coastal wetlands, and how targeting specific genes and metabolism can better help climate model efforts.

In the Appendix sections, I give an expanded characterization of the wetlands site description, hydrology, vegetation and topological heterogeneity. I observed that, although relatively close in geographical position, each wetland has a different salinity range, vegetation type and microtopography that can influence the distribution of microorganisms in the soil and water. Here, we also analyzed the redox potential, dissolved oxygen, pH and geochemical compounds (bromide, nitrate, ammonium, acetate, sodium, potassium, magnesium, sulfate, chloride, and iron (II)) of these wetlands. We found no correlation between geochemistry with depth, but noticed higher salt contents in the saltwater marshes, and shared geochemistry between the swamp and freshwater flotant wetlands, as expected. Conclusively, this thesis

contributes to the understanding of microbial communities to natural fluxes of methane in coastal wetlands and their interaction with the geochemistry of these ecosystems.

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CHAPTER 1: INTRODUCTION

1.1 Importance of coastal wetlands

Coastal wetlands are saturated ecosystems that can be micro-tidal or tidal, and have stream-flow into the ocean (Hopkinson et al., 2019). The ecosystem services and functions of coastal wetlands are wide and extensive. Coastal wetlands serve as natural storm protection, reducing the impacts of hurricanes on terrestrial lands (Constanza et al., 2008; Constanza et al., 2021). They also serve as natural habitat for a diversity of species of fishes, birds and mammals who spend at least part of their life cycle in wetlands (Pendleton, 2008). Because of their constant saturation and high organic matter content, wetlands maintain anoxic conditions that slow the decomposition of organic matter, and contribute to high levels of carbon storage (Nahlik and Fennessy, 2016).

Reports on global coastal wetland soils reveal an annual carbon sequestration rate of 18.7 t CO_2 e/ha/yr, with soil organic carbon averaging 825 t CO_2 e/ha in the top 1 meter of sea grasses, salt marshes and estuarine mangroves (Murray et al., 2011). When looking at the Gulf Coast wetlands, a report on freshwater, brackish and saltwater marshes, Cypress-Tupelo swamp and mangroves shows that this region can potentially accumulate 1,517 Gg of carbon per year, highlighting the importance of these ecosystems and different vegetation types to increasing carbon storage (Hansen and Janet, 2014).

One of the main challenges for the maintenance of ecosystem services in coastal wetlands is the sea level rise and consequent saltwater intrusion. Sea level rise rates in coastal Louisiana are 12±8 mm per year, one of the highest in the world (Jankowski et al., 2017). Saltwater intrusion has been positively correlated with the decrease in soil carbon storage because

saltwater contains ions that can cause mineral solubilization with increased release of nitrogen, phosphorus and silicon (Herbert et al., 2015). These ions also affect the microbial communities and mineralization pathways (Morrison et al., 2014). Saltwater intrusion also shifts environmental conditions that impact the interaction of established plant and microbial species, and negatively affect soil ecosystem functions (Herbert et al., 2015; Spencer et al., 2016). The implications of climate induced sea level rise on coastal wetlands remains an ongoing investigation, with respect to its impact on vegetation and soil accretion impacts (Grieger et al., 2020). However, there is a critical need for a broader examination that explores specific geochemical aspects of freshwater ecosystems affected by saltwater intrusion and their impact on methane cycling microbial communities.

1.2 Methane fluxes in wetlands

Recent estimates show that wetlands cover between 6% and 9% of terrestrial ecosystems, including tidal marshes, mangroves, swamps and floodplains (Constanza et al., 2014; Ramsar Convention on Wetlands, 2018). Despite covering relatively small areas, wetlands are major natural sources of the potent greenhouse gas methane and are predicted to exceed anthropogenic emissions in this century (Zhang et al., 2017). Uncertainties in predicting wetland methane emissions are due to the limited comprehension of environmental controls of methane production in these systems and need to be addressed in global climate models (Zhang et al., 2017; Bloom et al., 2017).

Methane emissions vary from wetland to wetland, depending on the water regime and vegetation covers (Zhiqiang et al., 2016; Vroom et al., 2022). Wetland plants have root traits that influence the production, consumption and transportation of methane through the soil by their aerenchyma, which are root and stem air channels (Iqbal et al., 2021; Määttä and Malhotra,

2024). Also, characteristic environmental conditions of coastal wetlands related to constant saturation, anoxic conditions, and pH extremes drive the selection of traits for plant adaptation and their participation in the methane emission process (Moor et al., 2017).

Because of their geographic location, which is often lower in elevation than other terrestrial ecosystems, coastal wetlands are susceptible to carbon and nutrient loading resulting from various anthropogenic activities, such as agriculture and industry (Valiela et al., 1990). Generally, this nutrient influx enhances plant productivity, which in turn, traps sediment on the soil surface around the vegetation, increasing sediment deposition and facilitating soil organic matter accumulation (Elsey-Quirk et al., 2019). Consequently, organic matter accumulation has been closely associated with the net methane fluxes in wetlands (Girkin et al., 2019).

Besides differences in water regimes and topography within wetlands, sea level rise and salinization of freshwater wetlands is still the main factor controlling the vegetation coverage in coastal wetlands (Morris and Sundberg, 2024). In coastal wetlands, research indicates that salinity intrusion initially leads to the collapse and death of freshwater vegetation (Middleton and David, 2022). However, as salt-tolerant plants are established in the system, there is a subsequent increase in above-ground production (Stagg et al., 2016). Some of these salt tolerant plants also produce exudates that further yield substrates used for methanogenesis (Waldo et al., 2019). For these reasons, salinity is still the most important controller of the methane cycle in wetlands and needs to be more investigated.

1.3 Salinity constraints on microbial methane cycling: ecophysiological perspective

From an ecophysiological perspective, the main constraints salinity brings on microbial communities are the osmotic imbalance salts cause in the system (Oren et al., 2013). The upper salt concentration limits which control microbial processes are variable. This means different

metabolisms impacting the methane cycling have different reported salinity thresholds. For instance, the energy conserving aerobic respiration has one the highest thresholds while these energetic processes typically have lower thresholds (Fig 1.1).

The major challenge for microorganisms living in saline systems is the need to maintain their intracellular salt concentration at least equal to the medium salt concentration. There are two ways microorganisms deal with an osmotic imbalance in saline systems: the salt-in and salt-out strategies (Oren et al., 2011; Oren et al., 2013). In the salt-in strategy, the cell can balance the osmotic pressure by accumulating inorganic ions internally. These salts are commonly chloride, sodium and potassium, and are used for enzymatic activities and protein structure. This strategy is a low-cost energy process, but leaves a narrow flexibility to environmental changes, as many microorganisms adapted to it can only function in high salinity systems. In the salt-out strategy, the cells pump salt out of the system to balance the osmotic pressure. This osmoprotection strategy is a much more energy demanding process, but it leaves more flexibility to the microorganisms across different salinity levels, since they do not rely on salts for their enzymatic functions (Oren et al., 2011). The latter is typically expected for wetland adapted microorganisms and especially those at the salt concentrations I observe in my study period.

Methane cycling microorganisms are especially affected by salinity stress. In saline systems, methylotrophic methanogens thrive, as they have more energy conserved to fuel the osmotic tolerance system. Alternatively, acetoclastic and hydrogenotrophic methanogens do not adapt to as high of saline concentrations, because of their inefficient osmoprotection system and their low energy generation per mol of substrate (Oren, 1999). Members of the methylotrophic methanogens are notable for having an efficient osmoregulation system that balances cytoplasm salt concentrations using the salt-in strategy outlined above (Bueno de Mesquita et al., 2021).

However there have been reports, some by our team, that show methanogens adapted to 150 g/L saline conditions (brine level) generate and take up osmoprotectants like glycine betaine (Borton et al., 2018). While this study does not examine the mechanisms of saline tolerance employed by methanogens, it does posit that methylotrophic methanogens will be more enriched in the most saline wetland samples, based on this ecophysiological established framework.

As for methane oxidizers, *Gammaproteobacteria* (type I) and *Alphaproteobacteria* (type II) respond differently to salinity stress based on their different membrane structure (Eshinimaev et al., 2002; Ho et al., 2018). For comparison, members of Type I methanotrophs have been favored by salinity stress, while type II methanotrophs were more negatively affected by salt stress. Saline tolerant methanotrophic bacteria can maintain an osmotic balance within the hypersalinity medium, either by synthesizing organic osmolyte solutes internally or by creating an extracellular organic protection (Trotsenko and Khmelenina, 2002). Both strategies keep salts out of the intracellular system. Studies show that wetland methane fluxes decrease with the introduction of salinity, and it could be related to the impacts it causes on methanotroph microbial communities (Holm et al., 2016). Admittingly, less is known about the responses of methanotrophs to saline conditions, a study area I focus on here. In summary, increased salinity affects methane cycling community diversity and composition based on the capacity of the microorganisms to gain sufficient energy to simultaneously support their energetic and osmotolerance strategies.

1.4 Salinity constraints on microbial methane cycling: ecological perspective

From an ecological perspective, salinity affects the methane cycling microbial communities by introducing substrates in the system that can increase microbial competition (Berrier et al., 2022). For example, sea level rise increases salinization and saltwater intrusion

also introduces sulfate (Kearns et al., 2016). The introduction of this electron acceptor can be used by respiratory sulfate reducing bacteria (SRB) for anaerobic respiration (Barton and Fauque, 2009), consequently affecting the structure of microbial communities (Kearns et al., 2016). This intrusion could therefore result in SRB outcompeting methanogens, as both compete for acetate (incomplete sulfate reducers) and hydrogen. The competition between SRB and methanogens is well documented in the literature (Lovley et al., 1983) and linked to the ability of sulfate reducers to effectively outcompete methanogens for substrates due to their lower half-saturation constant for these electron donors at *in situ* sulfate concentrations in saturated soils. Because SRB more efficiently compete for hydrogen substrates in saline systems, they grow more rapidly than the methanogens, further choking hydrogenotrophic and acetoclastic methanogens for substrate (Oren, 2011). While sulfate induced competition impacts both hydrogenotrophic and acetoclastic methanogens most, the sulfate reducing consumption of acetate is less energetically favorable and only encoded a subset of SRB that can completely oxidize acetate (Cui et al., 2017).

Similarly, salinity intrusion increases the availability of other substrates like iron and nitrate by changing the redox conditions of the soil environment and the mobility of substrates (van Dijk et al., 2015). These substrates can be used as electron acceptors by methanotrophs (Zhang et al., 2022). Interestingly, some methanotrophs can be associated with SRB in saline wetland systems, coupling oxidation of methane with the reduction of sulfate (Bhattarai et al., 2019). Anaerobic methane oxidizers (ANME) are archaea that can live in consortia with SRB in wetlands with high sulfate levels (Timmers et al., 2015). This association can control a large part of the methane cycle in wetland soils by reducing methane emissions (Zhang et al., 2022). Directly and indirectly, saltwater intrusion in freshwater wetlands affects methanogenesis and

methanotrophy, which climate models need to consider when being applied to create greenhouse gas mitigation plans.

1.5 Salinization alters the physicochemical nature of the soil environment

Beyond directly controlling methane cycling microbiota, coastal wetlands affected by saltwater intrusion face abiotic changes that can indirectly impact the microbiotic characteristics of the system (Breda et al., 2021). As noted above, saltwater intrusion in freshwater wetlands brings in sulfate and salts like chloride and sodium that can alter the soil physiochemistry. For example, sodium and potassium interfere with mineral bonding and dissolved organic matter contents of wetlands by causing flocculation or the dispersion of soil particles and their capacity to adhere organic matter (Wen et al., 2019; Wang et al., 2022). Furthermore, simulated experiments in coastal wetlands have shown that added salinity decreased the total soil nitrogen (Zhu et al., 2020). This happens because salts cause dissolubility of nitrogen from soil particles and consequent leaching, while also inhibiting denitrification (Strehse et al., 2018; Wang et al., 2022). Together this salinity induced change decreased overall carbon storage potential of the soils. The change in ionic strength fueled by salinity also contributes to remobilization and release of important nutrients used by native vegetation in wetland systems (Zhu et al., 2020; Hu et al., 2022). The vegetation shifts caused by salinity are further discussed in this thesis.

In addition to causing physicochemical changes in the soil system, mineral solubility caused by salinity intrusion can release toxic compounds to members of methanotroph communities (Zhang et al., 2023). Salinity causes functional and community inhibition among methane oxidizers in wetlands because it factors in water and cell surface loss (Li et al., 2022). More specifically, salinity disrupts the nitrogen cycle in wetlands, increasing ammonium availability in the system, which is a known inhibitor of methane oxidation (Guo et al., 2022). By

altering the biotic and abiotic properties of wetland soils and waters, saltwater intrusion can directly and indirectly affect the microbial methane communities and methane cycle in these systems.

1.6 Methanogenesis is impacted by salinity

Methanogenesis in wetlands varies as a function of the environmental conditions, substrate release from vegetation and microbial community composition (Zhang et al., 2018; Tiwari et al., 2020). The microbial community is especially important because methane is the metabolic product of anaerobic Archaea called methanogens (Robinson and Buan, 2018). Methanogens can reduce different growth substrates to methane gas, including carbon dioxide/hydrogen, formate, acetate, and methylated compounds in a process that synthesizes ATP (adenosine triphosphate) and allows for energy conservation (Schrink, 1997; Feldewert et al., 2020). There are three pathways for methanogenesis, and methanogenic archaea classified by their substrate use are called hydrogenotrophic (carbon dioxide/hydrogen, formate), methylotrophic (e.g. methylamine, methyoxy, methylated sulfur compounds), and acetoclastic (acetate) methanogens (Mand and Metcalf, 2019).

It is thought that salinity intrusion into freshwater wetlands impacts the methanogenesis pathways (Smith et al., 2018). Hydrogenotrophy is usually the main pathway for methanogenesis in these systems, as there is a relative abundance of hydrogen substrates and depletion of other electron acceptors like sulfate and iron (Lyu et al., 2018; Shima et al., 2020). Acetoclastic methanogens are less abundant in freshwater wetlands as acetate is a limited substrate and this pathway is less energetically favorable (Lyu et al., 2018; Stams et al., 2019). Studies show that saltwater intrusion in freshwater wetlands shifts the community from hydrogenotrophic to acetoclastic, because SRB are important competitors for hydrogen and grow under increased

salinity as described above (She et al., 2016; Berrier et al., 2022). Acetoclastic methanogens, however, do not often compete for hydrogen with SRB, as they use acetate instead (Berrier et al., 2022).

Methylotrophic methanogenesis is often considered the most active pathway for methanogenesis in highly saline wetlands (Kallistova et al., 2020). These methanogens are often regarded as having the highest salinity tolerance, and thus can grow without issues in saline wetlands by activating their osmoprotection systems (Oren, 1999). Besides their ability to tolerate saltwater systems, methylotrophic methanogens use non-competitive substrates such as methanol, methylamines and methylated sulfur compounds. Thus, methylotrophic methanogens are less constrained by increased sulfate with increasing salinity (Kurth et al., 2020). However, many of these theoretical "rules" have not been re-examined using more molecular based tools that allow broad sampling of the methane cycling microbiomes. Also, these theories are often derived from isolates or coculture testing in the laboratory and require broader testing under field relevant scenarios.

Prior research has established some constraints on methane production. For example, intermediate salinity levels (15%) significantly decrease plant productivity and methane fluxes, while high salinity (>30%) inhibits methanogenesis in tidal wetlands (Luo et al., 2019). These trends are especially observed in estuaries vegetated by less salt-tolerant plants. However, it is important to note that salt-tolerant plants can replace freshwater vegetation in wetlands affected by salinity intrusion in the long term, over time, and their exudates that methanogens, or their upstream carbon decomposing microbiota can use to generate methanogen substrates (Waldo et al., 2019; Yuan et al., 2019). My thesis begins to examine the microbiota and pathways of methane production in coastal wetlands located across a natural salinity gradient.

1.7 Methanotrophy in Wetland Systems

Methanotrophs consume or oxidize methane using it as an electron donor for respiratory metabolism. These microbes can either be bacteria or archaea (Wang et al., 2018). In wetlands, aerobic methane-oxidizing bacteria are generally the most active methane filters, consuming up to 60% of the produced methane (Singleton et al., 2018). Recent research into these lineages has established that many are not obligate methanotrophs, also participating in other ecosystem roles beyond methane oxidation, such as nitrogen and sulfur cycling (Bodelier et al., 2019). Methanotrophy also happens in anaerobic conditions, through the activity of anaerobic methanotrophic archaea (ANME), who can thrive in freshwater and brackish wetlands, and play a crucial role in the reverse methanogenesis process, where they use sulfate as an electron acceptor to oxidize methane, producing CO2 and water (Timmers et al., 2017; Zhao and Lu, 2023). Recent evidence suggests that canonical aerobic methanotrophs, while using oxygen to oxidize methane, can use nitrate to support growth, thus allowing methanotrophy to occur in much lower dissolved oxygen conditions than previously thought (Smith et al., 2019).

The environmental conditions affecting methanotrophy in wetland soils are similar to those affecting methanogenesis. Temperature, vegetation, soil pH, potential redox and salinity are some of the main drivers of methanotroph community abundance, composition and distribution in wetlands (Cui et al., 2020; Zhang et al., 2020; Van Grinsven et al., 2022; Hartman et al., 2024). In coastal wetlands, saltwater intrusion in freshwaters greatly affects the methane consumption by solubilizing ammonium, which inhibits methanotroph growth, and could increase the fluxes of methane to the atmosphere (Hartman et al., 2024). In this thesis, I also survey the methanotroph populations across freshwater wetlands trying to assess relative contributions of aerobic and anaerobic microbiota to this process.

In addition to methane consumption in the soils, it is increasingly being realized that methanotrophy in the oxygenated water column may be an important biological filter. Yet this is an under-investigated process, which constrains the development of accurate models for predicting global methane emissions (Bodelier et al., 2019). Most studies evaluating aquatic methanotrophs only focus on microbial communities in lake water columns (Iguchi et al., 2019; Rissanen et al., 2021; Venetz et al., 2023). Further, research from wetlands that couples sediment and water methanotroph communities is scarce. My thesis addresses a crucial knowledge gap, examining both the sediment and aquatic methanotroph communities, thereby advancing our comprehension of methane cycling in coastal wetlands.

1.8 Wetland vegetation impacts sediment wetland methane cycling

Wetland vegetation plays a significant role in shaping below ground microbial communities, with direct effects on microbial methane cycling (Figure 1.2). First, root-mediated effects are caused by root exudation and turnover from wetland plants, where sugars and organic acids are released into the surrounding soil (Jones, 1998). These labile carbon sources can themselves be substrates in the case of acetate or methanol or serve as substrates for other metabolisms which yield substrates for methanogens (Wolfe, 2005; Kurth et al., 2020). Moreover, the physical structure of plant roots can create anoxic microsites and contribute to soil aggregation, which provides suitable lower redox conditions for methane production (Lacroix et al., 2023). Secondly, wetland plants can control gas supply and transport. For example, some emergent species like *Typha* (cattail) and *Sagittaria*, act as gas conduits, transferring gas from soils in the roots through aerenchyma in the plant to the atmosphere, and vice versa from the atmosphere to the soil (Mo et al., 2015; Villa et al., 2020). This can enhance methane emission,

as methane does not diffuse through soils where it can be biologically oxidized by methanotrophs (Bodelier et al., 2019). Alternatively, it can constrain methane production, as this also transfers oxygen from the atmosphere to the roots, creating oxygenated zones around the roots that limit the methanogenic activity (Agethen et al., 2018). Thirdly, plant litter is an important source of the carbon and nutrients in the soils, and different vegetation contains different macromolecular concentrations, which can impact methane production. For example, sphagnum moss is a common component of peatlands, is high in polyphenols and other antimicrobials, and the litter itself acidifies soil. Together, this vegetative input can slow microbial decomposition in wetland soils (Chiapusio et al., 2018; Xue et al., 2023). In summary, the composition and diversity of wetland vegetation can impact soil methane dynamics by altering the availability of organic substrates, oxygen levels, and microbial community composition through root morphology, litter quality, and other rhizosphere processes (Bansal et al., 2020; Vroom et al., 2022).

In this thesis, while vegetation was not the primary focus of this work, I attempted to account for this impact on methane cycling microbial communities by sampling under different representative vegetative types where it was detected. This was to acknowledge any microbial community dynamics that could be attributed to land coverage differences. As such, you will see two vegetative patch types were investigated in the freshwater site, denoted Typha and Sagittaria, and two vegetative patch types in the saltwater site, Juncus and Spartina (see Appendix A). Additionally, methane fluxes were taken both above the vegetation to account for any plant production and consumption where aerenchyma plants were present, as well as on vegetation cleared soils, like in the unvegetated open water in the saltwater marsh and in the hummocks and hollows of the swamp.

1.9 A 16S rRNA gene sequencing paired with geochemistry approach to study methane cycling communities in coastal wetlands

The characterization of microbial communities in wetlands through sequencing the universal 16S rRNA gene can give us important information about environmental changes affecting biogeochemical cycles (Urakawa and Bernhard, 2017; Hall and Beiko, 2018). This gene is present in both archaea and bacteria in soils and waters, and has been used as a bioindicator for wetland monitoring (Quast et al., 2013; Sims et al., 2013). In wetland soils, this technique has been used to assess microbial community diversity, providing insights into complexity of microbial ecosystems, identify community composition by taxonomically profiling organisms, and in some cases distill ecological functionality, as many methanogens and methanotrophs can be identified by their taxonomy (Urakawa and Bernhard, 2017; Yarwood, 2018; An et al., 2019). Overall, 16S sequencing provides valuable information about the microbial diversity, composition, abundance, and ecological functionality, with potential applications to understand the methane cycle in wetland soils.

To evaluate the impacts of salinity on the methane cycling microbial community and their contribution to methane fluxes in coastal wetlands, I paired 16S rRNA gene sequencing data with methane flux, methane pore-water concentration, and geochemical measurements in soil and waters of Gulf Coast wetlands. I collected samples from depth resolved soils collected from cores and paired overlying aquatic samples. I sampled three coastal wetlands located in Louisiana that spanned a salinity gradient from freshwater to mesohaline (0.2 ppt – 13 ppt). Specifically, the two objectives of my thesis research were to:

1. Characterize the microbial contributions to methane concentrations in soil pore water and relate these to methane fluxes from coastal wetlands across a salinity gradient (Figure 1.3).

2. Examine the methanotroph communities in the sediment and overlying water to understand the methane buffering capacity in these samples (Figure 1.4).

Based on the existing theoretical framework, I hypothesized that acetoclastic and methylotrophic which compete less for substrates with sulfate reducing bacteria will be less impacted by salinity at the levels of membership and relative abundance than hydrogenotrophic methanogens, which I expect will be most negatively impacted by salinity (Pester et al., 2012; Conrad, 2020). Additionally, while less studied compared to the sediment methanotrophs, I hypothesized that oxygenated surface waters could contain methanotrophs. If these methanotrophs were consuming methane, supportive evidence would include that wetland sediments which have overlying water would have a greater decoupling between the methane production in the sediments and the total methane flux measured by chamber measurements. Together, the findings of this thesis can contribute to the understanding of methanogenesis and methanotrophy in coastal wetlands across a salinity gradient complex.

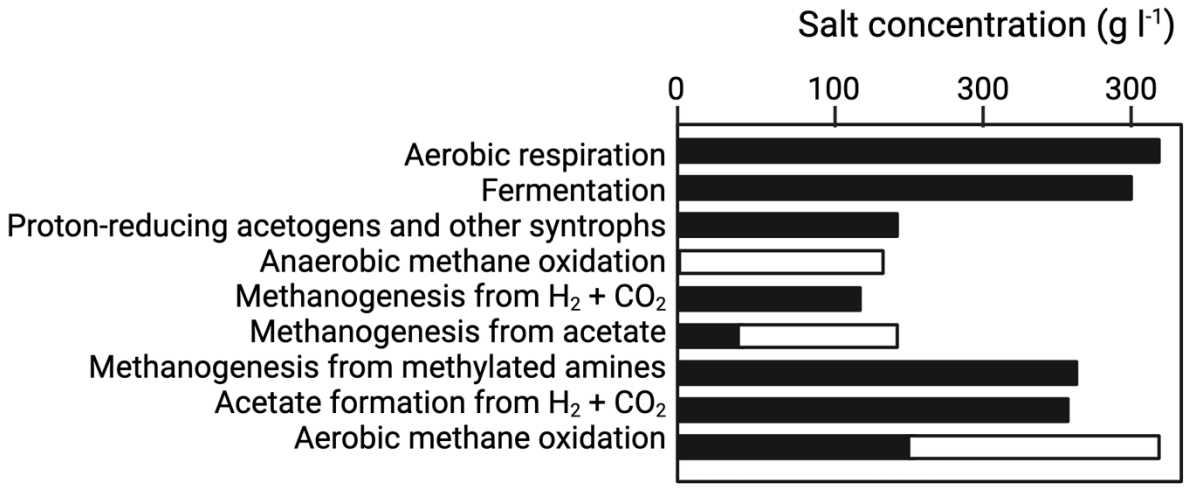

Figure 1.1. Approximate upper salt concentration limits for the occurrence of selected microbial processes valuable for the methane cycle in wetlands. Values presented are based on laboratory studies of pure cultures (black bars) and on activity measurements of microbial communities in hypersaline environments in nature (white bars). Note: this panel was derived from Oren (2011), (Copyright © Environmental Microbiology). 'Proton-reducing acetogens' refers to obligate syntrophic bacteria that produce acetate with the evolution of molecular hydrogen.

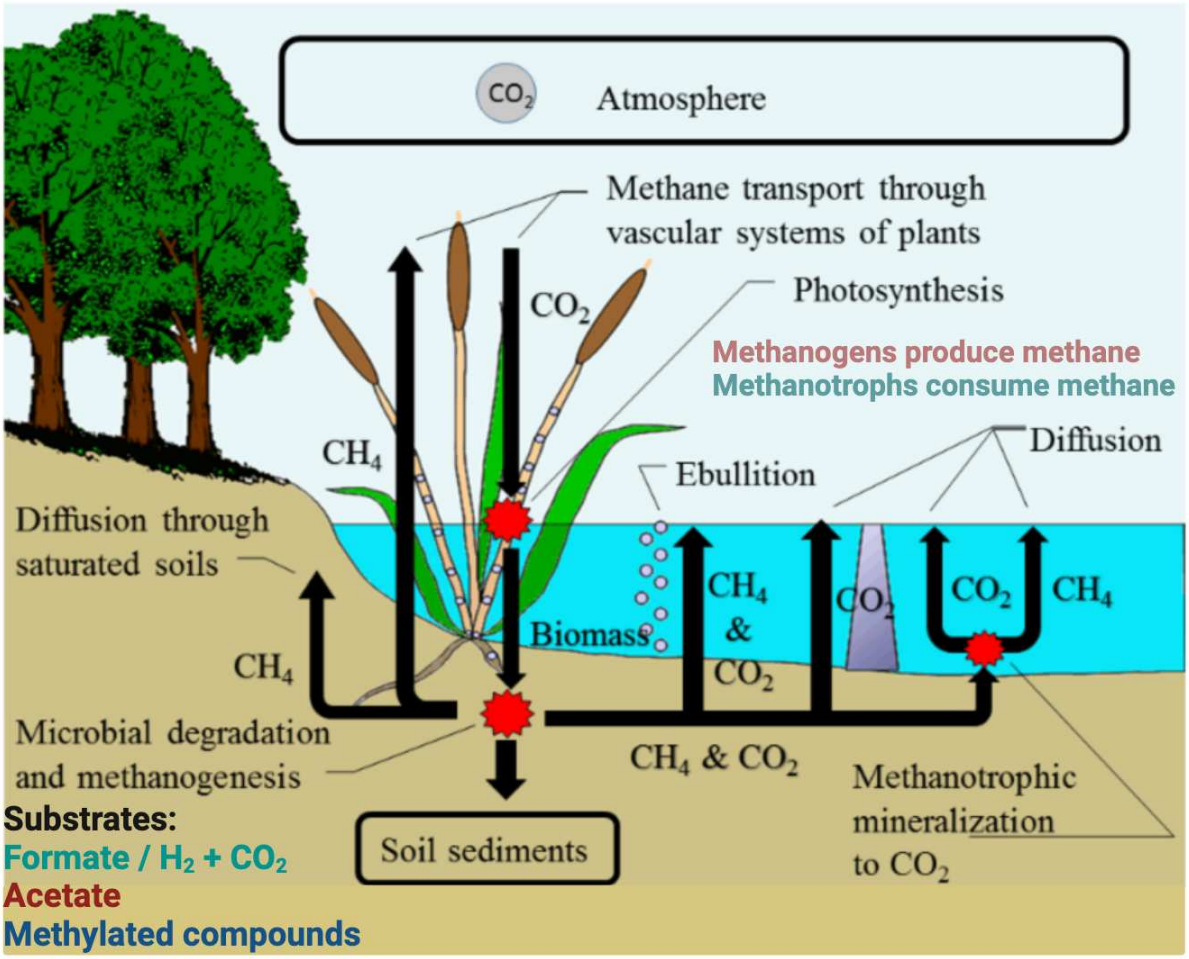

Figure 1.2. Graphical summary of the linkage between wetland soils and the atmosphere indicating entry and exit mechanisms including vascular systems of plants and the water column. Note: adapted from Timothy Morin, 2017.

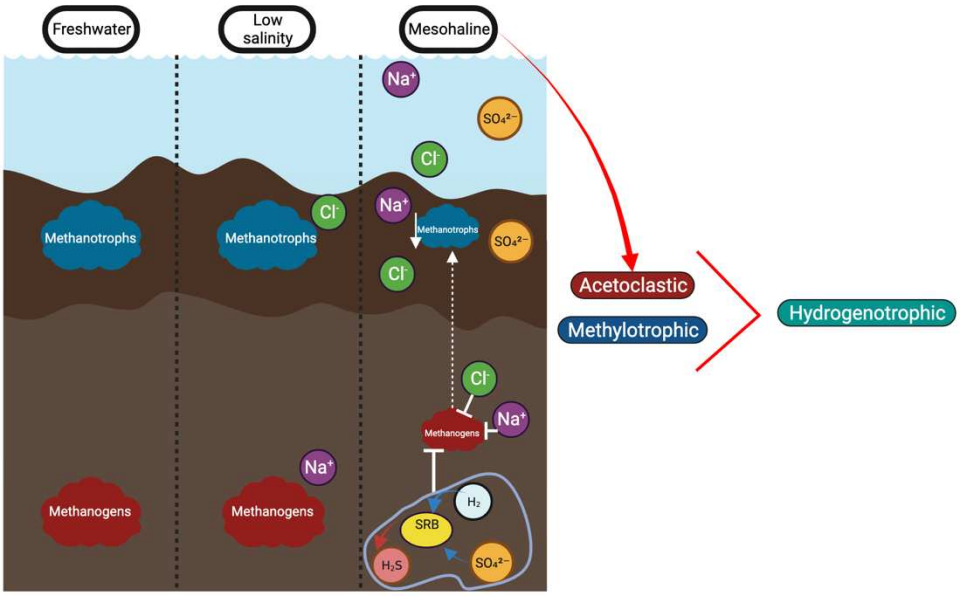

Figure 1.3. Scheme of existing theory suggesting that salinity can alter methane cycling microbial communities because (a) salts can be toxic to methanogens, especially hydrogenotrophic and acetoclastic lineages which are most sensitive than methylotrophic and (b) marine waters contain sulfate and harbor sulfate reducing bacteria, which compete for hydrogen more efficiently than methanogens thus further inhibiting methanogenesis.

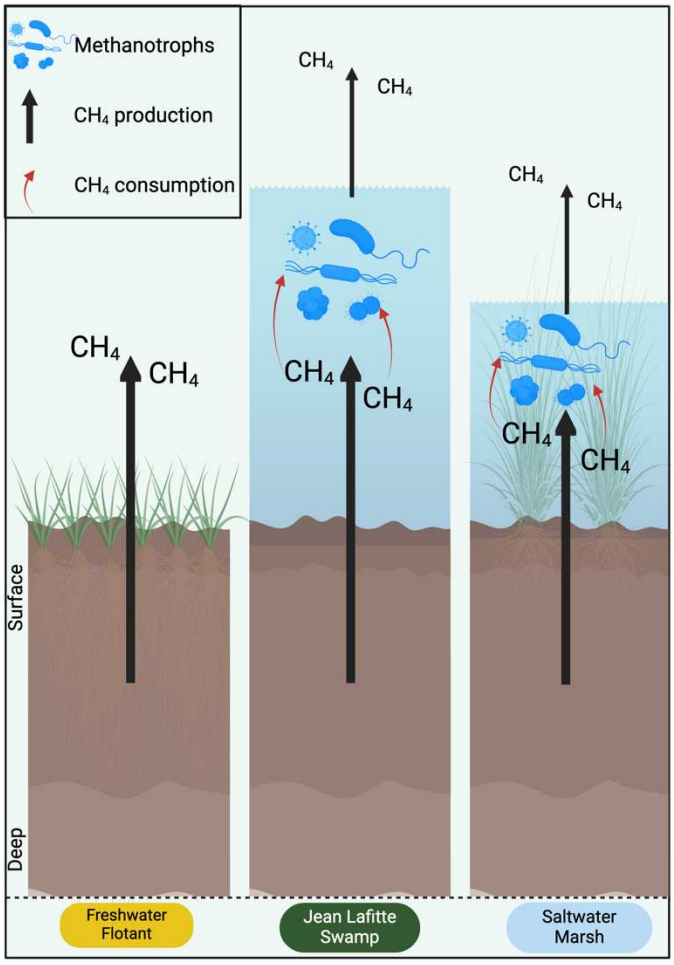

Figure 1.4. Scheme representing methanotroph communities in the water columns of Jean Lafitte swamp and saltwater marsh wetlands. Black arrows indicate methane production. Red arrows indicate oxidation of methane mediated by methanotrophs.

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CHAPTER 2: DECIPHERING THE BIOLOGICAL DETERMINANTS ON METHANE CYCLING FROM GULF COAST WETLANDS

2.1 Summary

Understanding the microorganisms and processes that contribute to methane fluxes in coastal wetlands is still limited, especially in saline systems which are less studied compared to their freshwater counterparts. Here I investigated the microbial and geochemical factors that contribute to methane emissions in three Gulf Coast wetlands located in Barataria Bay, Louisiana, USA. These wetlands had variable salinity, vegetation, and soil chemical characteristics. To understand the relationship between biotic and abiotic factors on methane emissions, I collected surface-level methane fluxes and paired this to sediment methane porewater concentrations, geochemistry, and 16S rRNA gene sequencing. I sampled three wetlands including a (i) freshwater flotant (0.1-0.2 ppt), (ii) low salinity Jean Laffite swamp (0.6-0.8 ppt), and a (iii) mesohaline salt marsh (6-13 ppt). I sampled multiple representative land coverage types (sites) at each wetland to account for observable differences in surface vegetation and topology, allowing for comparisons within and between wetland ecological "patch" types. Across these sites, the within wetland methane fluxes were not statistically different, thus I focus our discussion on the between wetland differences. Of the three wetlands, the freshwater flotant had the highest methane emissions, significantly more than the swamp and saltwater marsh which did not statistically differ. Soil and overlying water wetland microbial communities were structured by wetland type and depth (Figure B6, Appendix B). In the wetland soils, I observed the methane cycling microbial community richness and summed relative abundance decreased following the wetland salinity gradient. I did not observe changes in the relative abundance of

different methanogen functional types across wetlands, with hydrogenotrophs prevailing as the dominant type across all three wetlands. At the genus level, *Methanothrix*, an obligate acetoclastic methanogen, was dominant and cosmopolitan, detected in all wetland soils spanning the ecological sites and depths. I observed that soils with the highest in situ methane concentrations did not have the highest methane fluxes. Factors explaining this decoupling could be aerenchyma transport, as well as the high prevalence of methanotrophs in the oxygenated water column above low methane emitting soils. Together, these findings contribute to enhanced understanding of the microbiological contributions to methane fluxes in coastal wetlands.

2.2 Introduction

Methane budget estimations are underestimated in current global climate models, especially when wetlands are the methane sources evaluated (Yu et al., 2022). Coastal wetlands, important natural sources of methane, are particularly vulnerable to global warming and sea level rise, which increases uncertainty in predicting emissions (Liu et al., 2019). Understanding of the role played by microorganisms in wetland soils and overlaying water in terms of both methane production and consumption, as well as their interaction with environmental factors, remains relatively limited. Generally, salinity is reported as an inhibitor of methanogenesis (Xie et al., 2014; Luo et al., 2019). Yet it should be noted that biological methane production still occurs and is detected in fluxes from saline-intruded wetlands, likely because of microbial tolerance and temporal variation of environmental conditions (Helton et al., 2019; Wen et al., 2019).

Examining coastal wetlands differentially affected by salinity intrusion offers a natural laboratory for investigating the impact of salinity on methane cycling soil communities. Wetland salinity levels are classified as freshwater (<0.5 ppt), oligohaline (0.5-5.0 ppt), mesohaline (5.0-18.0 ppt), polyhaline (18.0 to 30.0 ppt), and euhaline for salinity levels higher than 30.0 ppt

(Montagna et al., 2013). Along this gradient, mesohaline wetlands are considered a critical salinity threshold, because at this level biogeochemical processes, like methane production, have been reported to decline (Wang et al., 2017). Similarly, other studies have shown that microbial organic matter mineralization pathways and rates also are negatively impacted both by shifting and decreasing at this salinity level (Weston et al., 2006).

The cycling of methane in wetlands is driven by microbial consumption and production (Lyu et al., 2018; Bodelier et al., 2019). In brief, the majority of methane is produced as a result of the reduction of carbon dioxide, acetate, or methylated compounds by anaerobic methanogenic archaea. Of the three main microbial pathways for methane production, hydrogenotrophic is usually the main pathway for methanogenesis in freshwater wetlands, followed by acetoclastic, while saline and hypersaline systems have been reported to be predominated by methylotrophic methanogenesis (Oren, 1999). Methane fluxes in natural systems are highly variable, depending on environmental conditions and land use changes across and within wetlands. This makes it necessary to understand the distribution of methane cycling microorganisms and their relationship with the geochemical characteristics (Rosentreter et al., 2021).

Large parts of this produced methane diffuse upward to the surfaces where it is oxidized to carbon dioxide in soils by anaerobic methanotrophic archaea (ANME) and eventually aerobic methanotrophs (Broman et al., 2020). Biological methane oxidation in wetland soils and sediments is considered an important filter, accounting for upwards of 20-60% removal of soil methane before it reaches the atmosphere (Singleton et al., 2018). Prior metagenomic studies from freshwater temperate wetlands have shown that bacterial members belonging to the order *Methylococcales* are likely responsible for majority of the aerobic methane oxidation in wetland

soils (Smith et al 2018). Compared to wetland sediments and soils, less attention has been paid to the methanotrophs in the oxygenated overlaying water column, and thus the methane buffering capacity of this compartment remains unrealized. Although methanotrophs play a key role in governing methane emission to the atmosphere in these climate critical habitats, the prevalent and dominant taxa, their distribution, and environmental factors controlling these populations are not fully understood in coastal wetlands.

To investigate the impacts of salinity on the methane cycling microbial community, I performed coupled geochemical analyses, greenhouse gas measurements, and 16S rRNA gene profiling on soils and water samples collected from 3 coastal wetlands (Figure 2.1). I compared soil from a freshwater flotant (n = 2 similar sites, 6 cores, 12 samples), low salinity Jean Lafitte swamp (n = 2 sites, 6 cores, 12 samples), and a mesohaline saltwater marsh (n=9 sites, 27 cores, 110 samples). Each of the 13 patch type sites was designed to account for the above ground heterogeneity within each site, accounting for the dominant land coverage type either vegetative (*Typha*, *Sagittaria*, *Juncus*, *Spartina*) or open-water (no dominant vegetation, but standing water), as well as micro-topographical formations (hummock and hollow) (Figure B1, Appendix A). Each site type was sampled in triplicate, with 3 cores at each site, for a total of 9 cores per site type. Each of these cores was sampled at two depths, a surface (0-10 cm) and deep (>30 cm) soil sample. In addition, of the 13 sites, 11 sites (all but those in the freshwater flotant) had standing water present, where I collected triplicate surface (0-5 cm) and deep (>30 cm) samples with a sipper resulting in 54 water samples (Figure 2.2). All soil samples had paired porewater methane emission, geochemistry, and microbiology data, while water samples had paired geochemistry and microbiology measurements. A summary table of every sample for microbiology and its paired geochemistry is included (Appendix C). Additionally, a table of the

emission data (Appendix C) and porewater greenhouse gas data for every sample is included (Appendix C).

2.3 Methods

2.3.1 Study site description and sample collection

In this study, a microbial and geochemical characterization of soil and water samples from three wetlands in coastal Louisiana-USA was conducted to analyze the patterns of microbial communities responsible for methane production and consumption under a salinity gradient. Samples were collected from a freshwater (0.1-0.2 ppt) flotant marsh (29°85'87″ N, 90°28'64″ W), low salinity (0.6-0.8 ppt) Jean Laffite swamp (29°80'18″ N, 90°11'02″ W), and a mesohaline (6-13 ppt) saltwater marsh (29°49'40″ N, 89°91'52″ W) (Fig. 2.2). The sampling campaign happened between the 26th and 29th of October of 2021, during peak methane fluxes (Holm et al., 2016). The dominant vegetation patches at each wetland are: *Typha* sp. (Site 1) and *Sagittaria* sp. (Site 2) in the freshwater flotant marsh, hummocks (Hu) and hollows (Ho) in the Jean Laffite swamp, and *Spartina* sp. (Sp), *Juncus* sp. (J) and unvegetated open water (O) in the mesohaline saltwater marsh. The water depth was higher at Jean Lafitte swamp (~65 cm), followed by the mesohaline saltwater marsh (~30 cm), while there was no water column above the freshwater flotant marsh peaty soils. Peepers were installed at each vegetation type for all three wetlands, and three cores of soil samples were collected at a maximum 2 meters distance around each peeper. Each core was sectioned into surface (0-10 cm) and deep (20-30 cm at freshwater flotant, 30-40 cm at Jean Lafitte swamp, and 40-50 cm at mesohaline saltwater marsh) depths, where three samples were collected per soil depth for microbial analysis. The water samples were collected at surface and deep layers of the water column of each wetland, and promptly filtered using MilliporeSigma™ Sterivex™ filters. Soil and water samples were

kept refrigerated in dry ice at the field, and later stored at -80 ºC for microbial analysis and at -20 ºC for geochemical analysis.

2.3.2 Methane porewater concentration and flux measurements

To measure methane porewater concentration, peepers with 20 stacked cells and 0.22-µm pore size polyethersulfon membranes (MacDonald et al., 2013) were installed in each predominant vegetation type patch of each wetland (n = 21). These peepers were installed at least one month before the sampling campaign to ensure equilibration. The extraction of 10 ml of water was conducted at every cell of the peeper until reaching 50 cm of soil depth. Samples were then placed in 10 ml sterilized tubes, where 0.2 ml of HCl 2 M was added to avoid biochemical changes of the gases collected. Samples were kept refrigerated until the moment of analysis. Methane porewater concentration was measured by a gas chromatograph headspace equilibration method (Kampbell et al., 1989), where a 5 ml subsample of each tube was equilibrated with 15-ml nitrogen headspace. From the equilibrated headspace, 10 ml was collected and transferred to a 10 ml pre-evacuated vial, then analyzed in the gas chromatograph (Shimadzu GC-2014, Shimadzu Scientific Instruments, Kyoto, Japan).

Surface methane flux measurements were conducted *in situ* in October 2021, using transparent 30 cm wide chambers of different heights depending on the water column and vegetation height, equipped with 12v fans at the base to better circulate gas to the analyzer. Each chamber had an inlet on the top that connected to a spectroscopy methane analyzer that recirculated the air at a rate of 1 l min^{-1} (Gas Scouter G4301, Picarro, Santa Clara, CA). Methane concentrations were recorded by the analyzer at 1-Hz frequency and each chamber deployment lasted 3 minutes. Three chambers for each vegetation type of each wetland (n = 21) were placed in static collars with dimensions of 30bcm x 30 cm x 15 cm, previously deployed into 2 cm

depth soil to ensure minimal disturbance of soils prior to the analysis. Chamber measurements included fluxes from soil and vegetation of vegetated sites. Methane porewater concentration and surface flux calculations are described in detail in Villa et al. (2020).

2.3.3 Geochemical measurements

Salinity levels were obtained from the Coastwide Reference Monitoring System (CRMS), an official federal program that monitors 390 Louisiana coastal sites using fixed sample schedule and standard data collection techniques (https://lacoast.gov/crms/#). Redox potential (ORP) was determined in situ using a portable Mv meter and redox electrode (Faulkner et al., 1989). Dissolved oxygen and temperature were determined in situ using a fiber optic oxygen meter (Fibox 4). Soil and water pH were analyzed with the Fisherbrand™ accumet™ AB150 pH benchtop meter. Ion chromatography (Dionex™ ICS-6000 DP) was used to determine anions (fluoride, acetate, chloride, nitrite, bromide, nitrate, sulfate and phosphate) and cations (ammonium, calcium, magnesium, potassium, sodium, lithium).

2.3.4 DNA extraction and 16S rRNA gene sequencing

Total nucleic acids were extracted from soil and water samples using the Zymo Research Quick-DNA™ Fecal/Soil Microbe Microprep Kit, following the manufacturer's protocol. The DNA quantity was determined using Qubit dsDNA HS Assay Kit, then stored at -80˚C until sequencing. The V4 region of the 16S rRNA gene using the F515 and R806 primer set (Caporaso et al., 2011) containing a unique sequence tag to barcode each sample was sequenced at the Center for Microbial Exploration at the University of Colorado Boulder on an Illumina MiSeq. The total reads were demultiplexed and analyzed using QIIME2 (2021.2.0) with DADA2, clustered with at least 99% identity, producing amplicon sequence variants (ASVs) that were then taxonomically classified using SILVA (silva132.250). Chloroplast and Mitochondria were

removed from the produced ASV table, and a subset of the ASV table was created to contain only taxa identified as methanogenic or methanotrophic, and methanogen taxa were grouped based on methanogen substrate use for statistical analysis. The relative abundance of each ASV was averaged over all samples for the whole microbial community and normalized to within each sample. For the methane cycling microbial communities, the relative abundance of each ASV was averaged over all samples, then summed by substrate use classification for methanogens, or aerobic/anaerobic classification for methanotrophs.

2.3.5 Taxonomic assignment of methanogens and methanotrophs

The ASVs were linked to taxonomic string using SILVA (silva132.250), and then manually classified as methanogenic and methanotrophic via the known physiology of the most resolved taxonomic level for each (Guerrero-Cruz et al. 2021, Lyu et al. 2019, Evans et al. 2019). All ASVs classified as methane-metabolizing were found to at least belong to known methanogenic or methanotrophic orders (Guerrero-Cruz et al. 2021, Lyu et al. 2019, Evans et al. 2019). Membership to typically considered methanogenic or methanotrophic classes was not considered sufficient, as one ASV here assigned to a typically methanotrophic class represented a likely known non-methanotroph (Ivanova et al. 2021). Where possible, ASVs were preferentially most confidently assigned as methanogen or methanotroph based on genus level taxonomy. However, for ASVs representing novel or uncultured taxonomic strings, order or family-level taxonomy was relied upon only if it represented the most resolved unit of taxonomy for the ASV. Members of the *Syntrophoarchaeaceae* were classified as likely alkanotrophs per Evans et al (2019) and were included with the methanogenic group for this study.

2.3.6 Statistical analysis and data visualization

The statistical differences in comparison of methane porewater concentration and fluxes between wetlands were calculated with ANOVA and Tukey's Honest Significance Difference (HSD) using the rstatix (Kassambara A, 2023) package in R (4.2.0). The final methane cycling microbial ASV table was curated to have the lowest taxonomy level of each ASV for statistical purposes. The relative abundance of microbes and alpha diversity (Shannon's index) were calculated using the phyloseq (McMurdie and Holmes, 2013) package in R (4.2.0). To explore the microbial community beta diversity, a non-metric multidimensional scaling (NMDS) analysis measured with Bray-Curtis dissimilarity index was conducted and compared to a principal components analysis (PCA) of geochemical measurements (pH, salinity, anions and cations). To identify statistical differences between methane cycling microbial communities and geochemical measurements among wetlands, an analysis of similarities (ANOSIM) and multi-response permutation procedure (MRPP), as well as permutational multivariate analysis of variance (PERMANOVA) were conducted comparing wetland type, dominant vegetation, depth, and sample type. The top 10 geochemical compounds driving differences in ordination were based on the distance in space from the center of plots. All beta-diversity statistical analyses were performed using the vegan (Oksanen J, et al., 2022) package in R (4.2.0). Basic plots were generated using the ggplot2 (Wickham H, 2016) package in R (4.2.0), and further illustrated using the BioRender software.

2.4 Results and Discussion

2.4.1 Methane fluxes but not soil production follow an expected salinity gradient

I first examined the methane emissions within each site. Within the freshwater flotant site, the site level differences were not significant. This is not entirely surprising given the two

sites had similar vegetation types, and were both dominated by *Typha* sp. and *Sagittaria* sp. Within the swamp, I did not see significant differences between hummocks and hollows. I accounted for these microtopological differences as prior reports suggested that these differences could influence lateral runoff resulting in increased soil organic carbon concentrations, which can be associated with different fluxes within the same wetland (Aleina et al., 2015). However, prior reports from methane fluxes from peatlands with the same micro-topographic gradient as our swamp site echoed these findings, with no statistical difference in microtopographical differences in methane emissions (Villa et al., 2019).

Lastly, the saltwater marsh was composed of three distinct land coverage types (unvegetated open water, *Juncus* sp., and *Spartina* sp.). Within this marsh I did not detect significant differences in methane flux, yet there was a general trend that the lowest methane fluxes across all the sites in our three wetlands was from the open water sample. This finding is supported by a prior study showing that open water samples with low to no detectable vegetation cover had decreased methane fluxes in coastal wetlands (Chen et al., 2018). However, our finding that microsite heterogeneity was not a primary influencer of methane emissions across 3 wetlands is only from a single time point, and warrants further temporal investigation. Despite this limitation, the implications are large, as it may not be necessary to partition wetland biogeochemical models by ecological regimes in some coastal wetlands, as some have suggested (Vizza et al., 2017).

Given there was no within wetland site effect, I tabulated the mean fluxes from all sites in a wetland and then compared this mean wetland value across wetland salinity gradient. Here, methane fluxes decreased along with increasing wetland salinity (Figure 2.3A). The freshwater wetland emitted the highest fluxes, followed by the swamp, while the wetland with the greatest

salinity (the saltwater marsh) had the lowest overall fluxes. Methane fluxes from the freshwater wetland were statistically increased relative to the both the swamp and saltwater ($p<0.05$). There was no significant distinction between the fluxes from the swamp and saltwater wetlands.

While I did see a pattern in methane fluxes decreasing with increased salinity, methane porewater concentration among wetlands did not follow the salinity gradient (Figure 2.3B). I found methane production in soil by averaging the methane concentrations from each depth measurement (0-10 cm, 20-30 cm, 30-40 cm, and 40-50 cm, n=4). The mean 44.38 μM of methane measured in the freshwater soils significantly differed to an average of 500.62 μM in swamp and 311.88 μM in saltwater marsh, with no significant difference in in situ concentrations from the later wetlands. I observed a decoupling between the methane concentrations in the pore water and the overall chamber emission data, as the highest methane producing soils (swamp wetland) had the second lowest methane emissions. Likewise, the freshwater flotant had the lowest concentration of methane across all soil depths (almost tenfold less than the other sites) yet had a higher soil emission.

This decoupling between wetland soil methane concentrations and fluxes has been reported before (Bansal et al., 2020). Low soil methane concentrations and higher fluxes can be associated with plant-mediated transport of methane through aerenchyma, where higher emissions of methane were observed directly over plants rather than in bare soils. However, that study also reported lower methane porewater concentration below whole-plants in comparison with the bare soils (Bansal et al., 2020). This may be a possible explanation for the freshwater wetland in our study, where aerenchyma containing *Typha* and *Sagittaria* plant species dominated. We did not observe this decoupling in the saltwater marsh vegetated sites, which are dominated by *Juncus* and *Spartina* plant species, despite these plants also having aerenchyma

transport. However, plant species have distinct aerenchyma transport mechanisms. For example, *Typha* use pressurized flow that can contribute to higher gas transport rates than diffusion alone. Beyond transport mechanisms, the total methane emission through a wetland vegetation is also influenced by growth stage, water table depth, balance between sediment and in-plant methane concentrations (Vroom et al., 2022). Thus, I consider it possible that efficient aerenchyma transport in the vegetation from the flotant marsh, could have contributed to lower measured soil porewater methane concentrations and higher observed methane fluxes at this wetland. For the other sites, where the decoupling is reversed with higher soil concentrations and lower fluxes, I consider it possible that methanotrophy in the oxygenated water column could play a role in methane buffering (Venetz et al., 2023).

Some studies measuring along wetland depths sampled similarly to our experimental design have reported increased methane concentrations at deeper depths. This could be because deeper soils are typically the most anoxic and deprived of other electron acceptors that allow respiratory microorganisms to outcompete methanogens (Arnold et al., 2023). By comparing the methane concentrations from each peeper (n=39) at 10 cm intervals, I did not observe a single depth that consistently had the highest methane concentration (Figure 2.3). For example, the highest methane concentration in the freshwater flotant was observed at 40-50 cm for both Sites 1 and 2, while the 20-30 cm depth was the highest in the hummocks of the swamp wetland, but not for hollows, where the 30-40 cm depth had the highest methane concentrations. In the saltwater wetland, both open water and *Spartina* sp. sites had the highest concentrations at the lowest depth (40-50 cm), while *Juncus* sp. methane concentrations were higher at 20-30 cm. This finding may illustrate that within and across wetlands, microbial communities change more by

cm-scale depths rather that laterally by hundreds of meters as others have suggested (Narrowe et al., 2017). This has implications for future microbial sampling across wetland gradients.

2.4.2 Methane cycling microbial diversity and composition are structured by wetland type and depth

In total, 105 soil and 41 water filter samples were collected and were DNA extracted and 16S rRNA gene amplicon sequenced. A total of 8,284,621 high-quality reads were generated for the 146 samples. After data processing, I identified a total of 60,605 ASVs with a matching sequence of at least 99% identity, which were then taxonomically assigned using Silva. Of these, 709 could be taxonomically identified as belonging to methanogenic or methanotrophic communities using the definitions and descriptions outlined in Appendix C.

I compared alpha diversity across the wetlands for both the entire microbial community and the subset of methanotroph and methanogen (methane cycling) members. In each wetland, the mean microbial richness per sample was 1316, 1177, and 1206, respectively for freshwater, low and intermediate salinity, and there were no statistical differences by wetland (Figure 2.4A) or depth. As expected, diversity followed the richness data showing a similar lack of wetland wide significant differences. However, when I performed the same analysis for the methane cycling (methanotrophs and methanogens) members, I did find that methane cycling richness and diversity decreased along the salinity gradient.

Consistent with this finding, inhibitory effects of salinity are reported to control the distribution of methanogens (Webster et al., 2015) and suppression of methane production (Luo et al., 2019) across estuarine wetlands even at lower salinity ranges like thoseinvestigated here. I was surprised to see the salinity had a more clear impact on the methanotrophs diversity than the methanogens (Figure 2.5AB). It is not known whether this impact is direct toxicity of the salt, or

an abiotic effect of salinity solubilizing ionically bound ammonium, which inhibits methanotrophs (Hartman et al., 2024). Similar findings have also been reported from inner wetlands and lake sediments (Zhang et al., 2022). Our findings show that salinity could be a potential influencer on the diversity of methane cycling microorganisms.

In our sites, soil microbial communities both at the whole community level (Figure 2.6) and methane cycling community level (Figure 2.7) were statistically differentiated by wetland. The saline wetland communities were most distinct and separated along axis NMDS1, while the low salinity swamp and freshwater flotant had more similar membership but were still statistically different along axis NMDS2. Next, I used Permutational multivariate analysis of variance (PERMANOVA) to identify the geochemical factors that best explained the clustering observed in our microbial community data. Along axis 1, salinity and variables influenced by salinity (e.g., chloride (Cl-), magnesium (Mg), and fluoride (F-)), were the largest drivers of community differences between the salt marsh and the flotant and swamp. Across all wetlands, depth or related variables (Fe(II)) was the biggest universal driver of microbial community membership, impacting both the entire community and methane cycling communities as shown in NMDS axis 2 ($R^2 = 0.994$, $p = 0.001$).

2.4.3 Methanogen functional type is not impacted by salinity

Based on the differences observed in the NMDS, I next investigated the methanogen genera and their relative abundance across the surface and deep samples from each wetland site. I assigned these wetland genera to functional substrate groups based on established knowledge derived from genome analyses of representative members (Figure 2.8A). *Methanothrix*, an obligate acetoclastic methanogen, was a core, dominant methanogen in both surface and deep soils from each site, as it was found in at least 80% of all samples across wetlands, sites, and

depths. Previous reports have shown that *Methanothrix* could have global importance in the wetland methane cycle, as it is widely distributed across different freshwater wetland types and equally abundant in surface and deeper soils (Angle et al., 2017, Chen et al., 2020), corroborating our findings observed here. Also in coastal wetlands, members of this genera have been reported to be saline-tolerant (Li et al., 2023). Along these lines, studies have identified the widespread distribution of this genus across coastal wetlands like mangroves, salt marshes, and seagrasses globally, affirming its resilience to varying salinity levels (Cai et al., 2022).

While a single acetoclastic methanogen genus was dominant across sites, when we totaled the relative abundance of all the methanogen ASVs to their functional guilds, hydrogenotrophic were the most dominant, accounting for 15% of the methanogen community relative abundance (Figure 2.8B). This finding contrasted with my expectation that hydrogenotrophs would decrease with salinity, either because the salinity was directly toxic, or competition with SRB enriched by higher sulfate concentrations. I note this response could be because we summed the relative abundances of many, rare taxa (n=154), as these rare members may not be impacted by salinity. Along these lines, I did not observe a single hydrogenotrophic genus that was universally abundant across or even within a wetland, indicating greater phylogenetic diversity within this functional guild compared to acetoclastic guild. Hydrogenotrophic methanogens were the most discriminant between wetlands with *Methanomicrobiacae* most discriminant for the saltwater marsh, while 8 other genera discriminated the freshwater flotant and 5 genera in the swamp (Figure 2.9). Unlike the acetoclastic methanogens that had a dominant genus conserved across wetlands, or the cumulatively abundant hydrogenotrophs, the methylotrophs did not show clear wetland enrichment.

This finding of the methylotrophic methanogens not responding to salinity contrasted with the existing theory that methylotrophs are enriched in response to salinity. These taxa are typically more salt tolerant (Kallistova et al., 2020) and saltwater plants like *Spartina*, one of the plants we studied here, can exude trimethylamine supporting methylotrophic methanogens (Yuan et al., 2019). These lack of functional type differences and methylotrophic enrichment was not due to lower saline range of our highest saltwater marsh, as other mesohaline wetlands have exhibited such patterns. For example, a study conducted in a mesohaline saltmarsh with salinity ranging between 6.6 to 14.5 ppt described the prevalent co-occurring of methylotrophic methanogens along the marsh during various seasons, being again correlated to the life cycle of *Spartina* sp. in these sites (Capooci et al., 2023). My findings indicate that these "rules" on methanogen type and distribution across wetlands likely need further scrutiny and may not be universal by wetland salinity. I suggest that other underappreciated factors like vegetation, salinity perturbation frequency, or other chemical factors may instead be the yet defined influencer of biogeographic patterns of methanogens across coastal wetlands.

2.4.4 Methanotrophs abundance is increased in surface waters

The relative abundance of methanotroph communities in soils across wetlands decreased from the freshwater flotant to the saltwater marsh, thus following the salinity gradient, and supporting my hypothesis that salinity would decrease methanogen abundance (Figure 2.5A). In addition to variations in the relative abundance of methanotrophs across wetlands, I noted that methane oxidizers exhibited more site-specific diversity compared to methanogens. Few methanotroph taxa were shared among different wetlands, and no core members were found between them (Figure 2.9). Cumulatively across the wetlands aerobic methanotrophs, especially those from genera within the Gammaproteobacteria (*Crenothrix*, *Methylocistis*, and

Methylomonas) and Hypomicrobiaceae (*Methyloligellaceae*, *Methyloceanobacter*) were dominant in these wetlands.

The only anaerobic methanotrophs (ANME-1b) were found in the saltwater marsh wetlands, representing dominant members of the soil methanotroph community. Anaerobic methane oxidation (AOM) is a low energy process, and members of ANME are adapted to saline low hydrogen-high sulfate wetlands, where they can outcompete other methanotrophs (Kevorkian et al., 2021). Other prevalent taxa found in the mesohaline wetlands were *Methyloceanibacter* (38.25%), and *Methyloligellaceae* (16.42%). These findings show that despite the overall decreased diversity of methanotrophs, saline wetlands still have methanotroph taxa that can likely play an important role in buffering wetland methane emissions.

Given that I observed high methane pore water concentrations but low overall fluxes in our saline impacted swamp and saltwater marsh sites, I hypothesized the overlaying water column only present in these sites could be acting as biological filter. Specifically, I evaluated the relative abundance of methanotrophs at surface (0-10) and deep (~30 cm) sites within the 65 cm deep water column within the swamp and 30 cm deep water column within the saltwater marsh. Consistent with their possible role as biological filters, I detected methanotrophs in the water column, especially at the surface samples (Figure 2.11). I found that surface water methanotrophs were more abundant and distinct from the taxa found in soil within the same wetlands, but similar taxa were shared between waters of the swamp and mesohaline wetlands (Figure 2.12). *Methylomonas* was the dominant aquatic methanotroph taxa shared between the two wetlands, accounting for approximately 25.63% of the methanotrophic community in the water columns. Interestingly, *Methylomonas* was not detected in the underlying soils of these wetlands.

Despite the importance of methanotrophs as a biological filter, these taxa are rarely considered in the water column, but instead focused on the soils. In fact, few studies sample across both the water column and soil profile. Recently, some studies exploring methanotroph methane removal capacity in the water column have been conducted in lakes (Rissanen et al., 2021; Su et al., 2023; Żygadłowska et al., 2023), although not comparing them to soils of the same wetlands. Next steps would include determining these lineages were metabolically active and contributing to isotopic fractionation of the methane as it traveled across the terrestrial to aquatic compartments. The variability among methanotroph taxa in soil and water systems of the same wetlands underscores the necessity for further exploration of the contributions of these microorganisms to the methane cycle.

2.5 Conclusions

Here I characterized soil and water samples in three wetlands along a natural salinity gradient from freshwater marsh, low salinity swamp, and a mesohaline saltwater marsh. While accounting for micro topographical and vegetative differences within a wetland, I observed these ecological types did not result in statically different methane emissions. Instead, I did observe across wetland methane production and fluxes variation. In the case of methane fluxes, I observed decreasing relationship to increased wetland salinity. I consider it possible that this relationship to salinity impacted the methanotrophs, which had a stronger observed decreased diversity with salinity, and were more responsive to specific wetland conditions. In comparison, I observed a single obligate acetoclastic methanogen was the dominant genus across wetlands, but hydrogenotrophic methanogens were the most cumulatively numerous functional guilds across every wetland. Methane emissions within coastal wetland systems revealed a decoupling, where high porewater concentrations were not reflective of high fluxes, and vice versa. I posit that

methanotrophs in the water column and differences in aerenchyma transport mechanisms could be contributing to this decoupling, respectively. In conclusion, salinity or its related chemical conditions (sulfate, chloride, ammonium) is an important environmental factor that could shape methane cycling microbial community abundance and composition across wetlands. It does not appear to affect porewater methane production, but future multi-omic and isotopic studies are needed to further evaluate this supposition.

Understanding the distribution and controls on microbial methane production from coastal wetlands soils is critical to global methane emission predictions, particularly considering changing environmental conditions. Here I show that hard and fast rules about the biogeography of methanogens along salinity gradients (e.g., enriched methylotrophs, negatively impacted hydrogenotrophs) warrant investigation across a greater number of coastal wetland systems. These results highlight the need for examining the entire terrestrial-aquatic column, and not considering any one compartment in isolation. It is our goal that this biological framework can be used to generate knowledge base for increased realism in predictive, process-oriented models on methane fluxes.

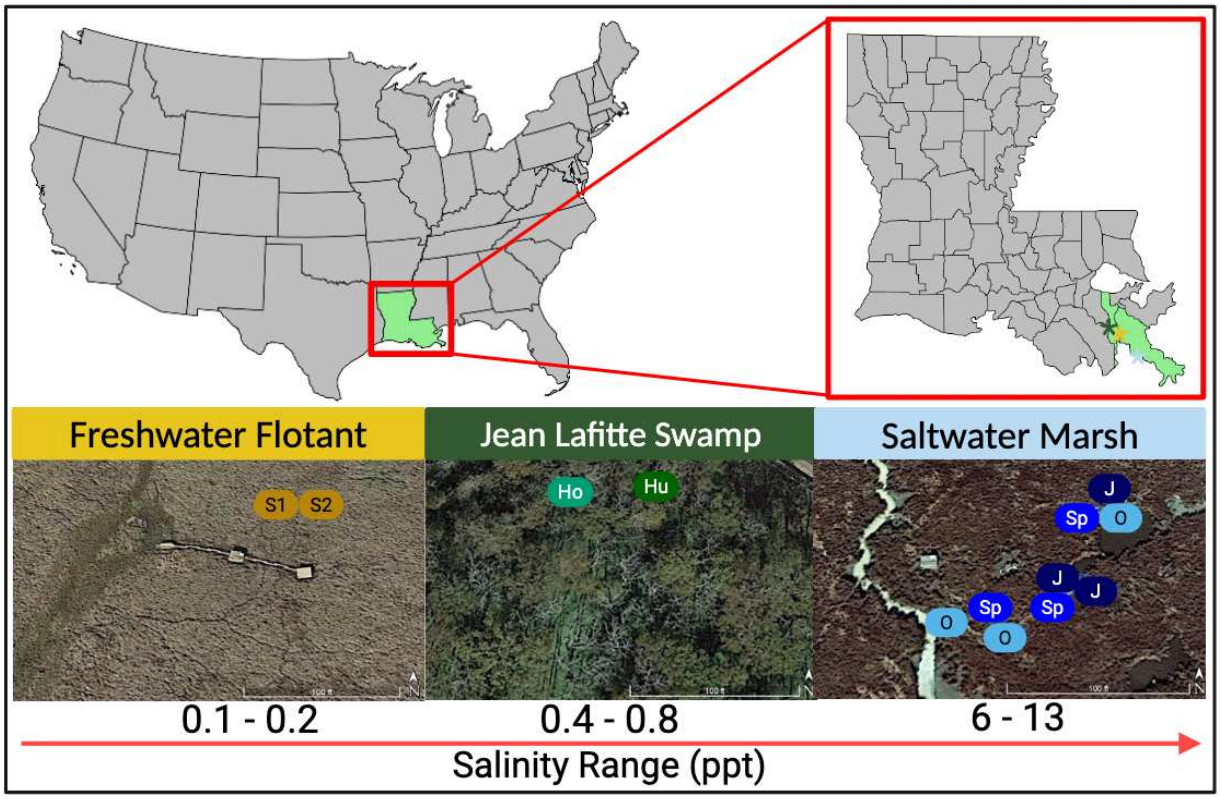

Figure 2.1. Map of coastal wetlands and site locations following a salinity gradient ranging from 0.1-0.2 ppt in a freshwater flotant, 0.4-0.8 ppt in a low salinity swamp, and 6-13 ppt in a saltwater marsh in Barataria Bay, Louisiana, USA.

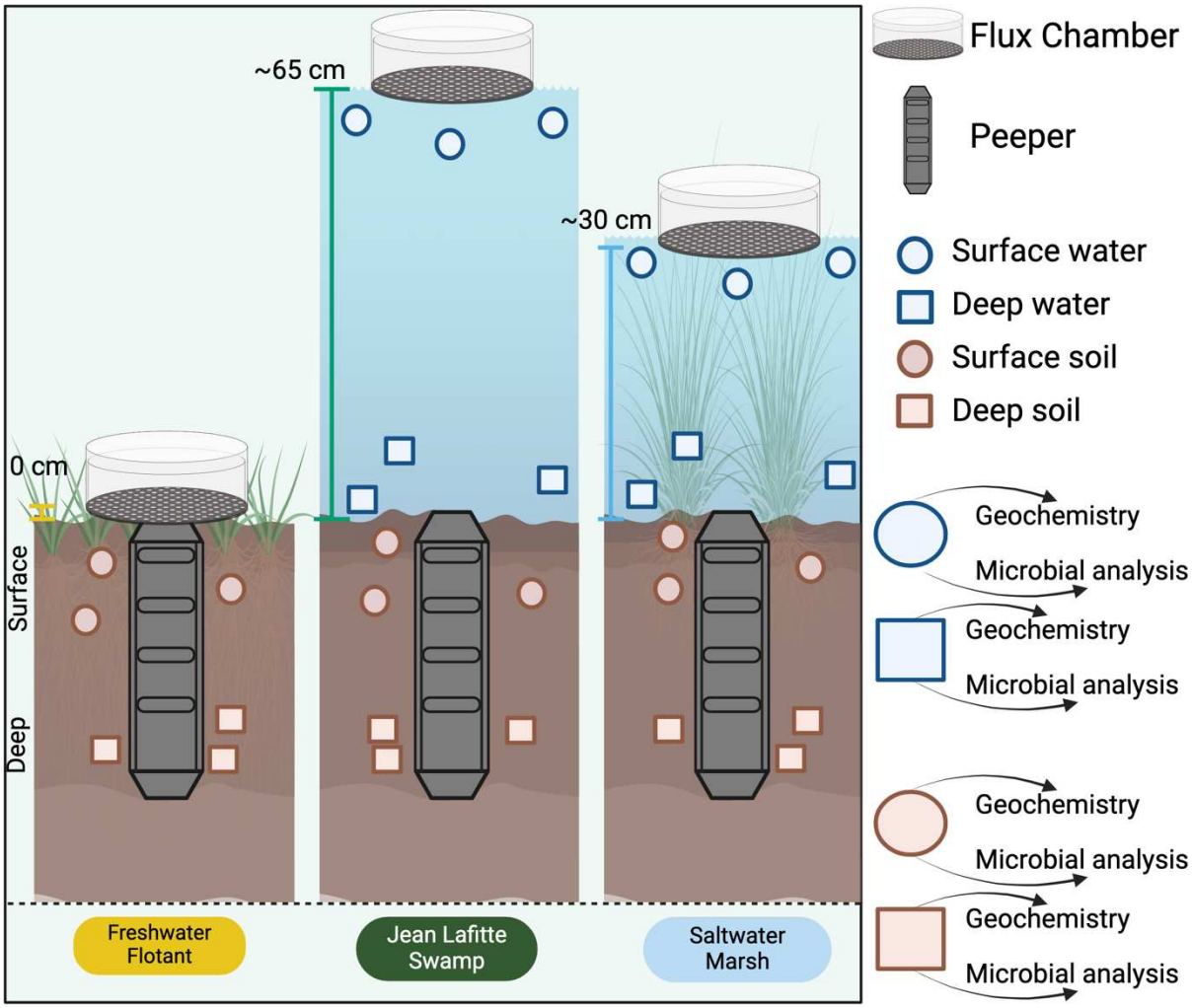

Figure 2.2. Schematic experimental design sampling collection for each site. Each site was denoted by having a pore-water dialysis (peeper) for measuring in situ methane and carbon dioxide concentrations along soil depths. Chamber measurements were used to measure carbon greenhouse gas fluxes from the sites. Triplicate surface and deep soil samples and water filters were collected from each site. Each sample has paired geochemical and microbial analysis, with soils also have porewater methane from the peepers. Field sampling was conducted in October 2021, during peak methane emissions.

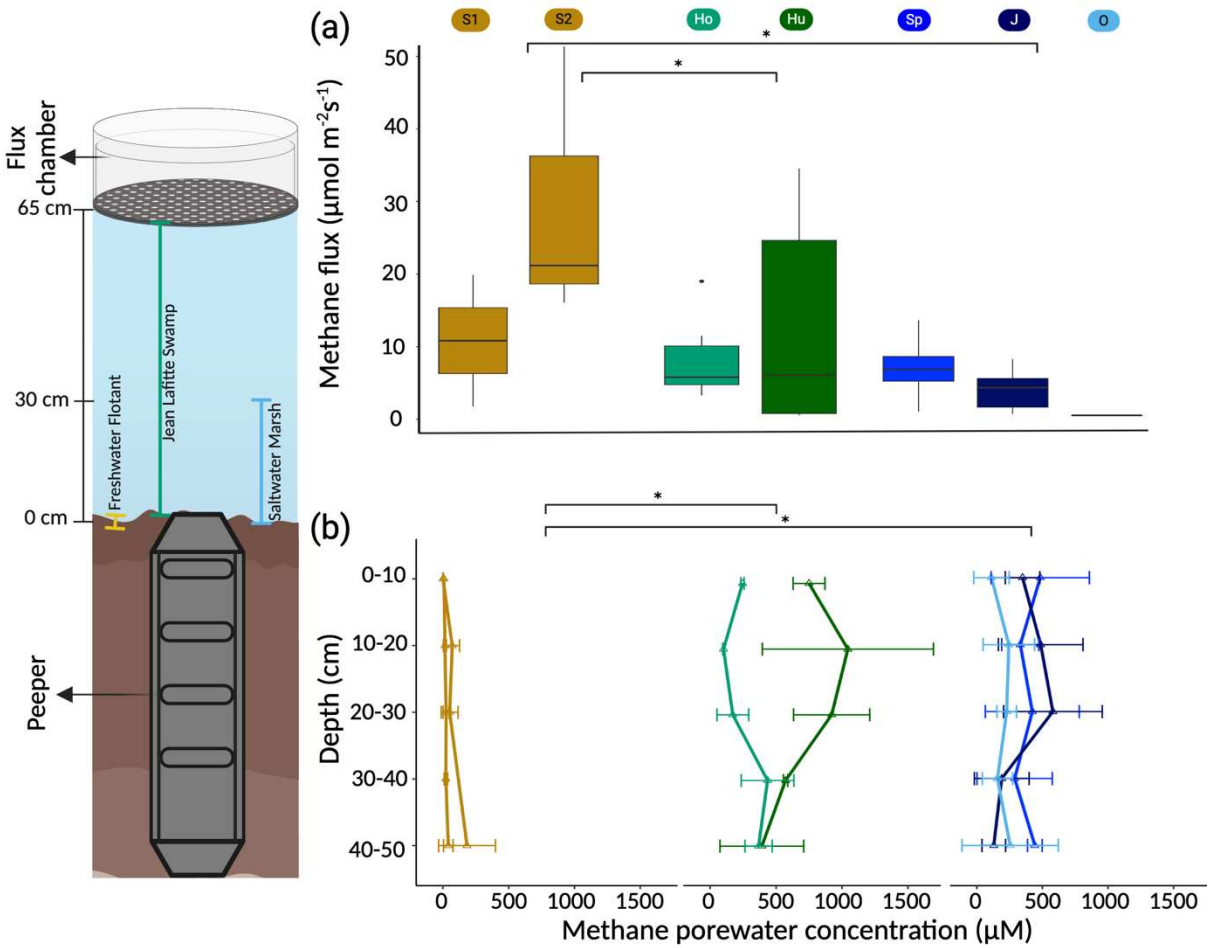

Figure 2.3. Overall schematic of sampling across the three wetland sites. (a) Flux chambers were used to collect methane fluxes and (b) peepers were used to collect methane porewater concentration in all sites. Boxplots represent the median (horizontal black line inside the box), lower and upper quartiles (25% and 75% of interquartile ranges), ranges (5% and 95%, vertical lines) and outliners (black dots) for each diversity measurement. Brackets are comparing richness and Shannon Index between wetland types (*p<0.05).

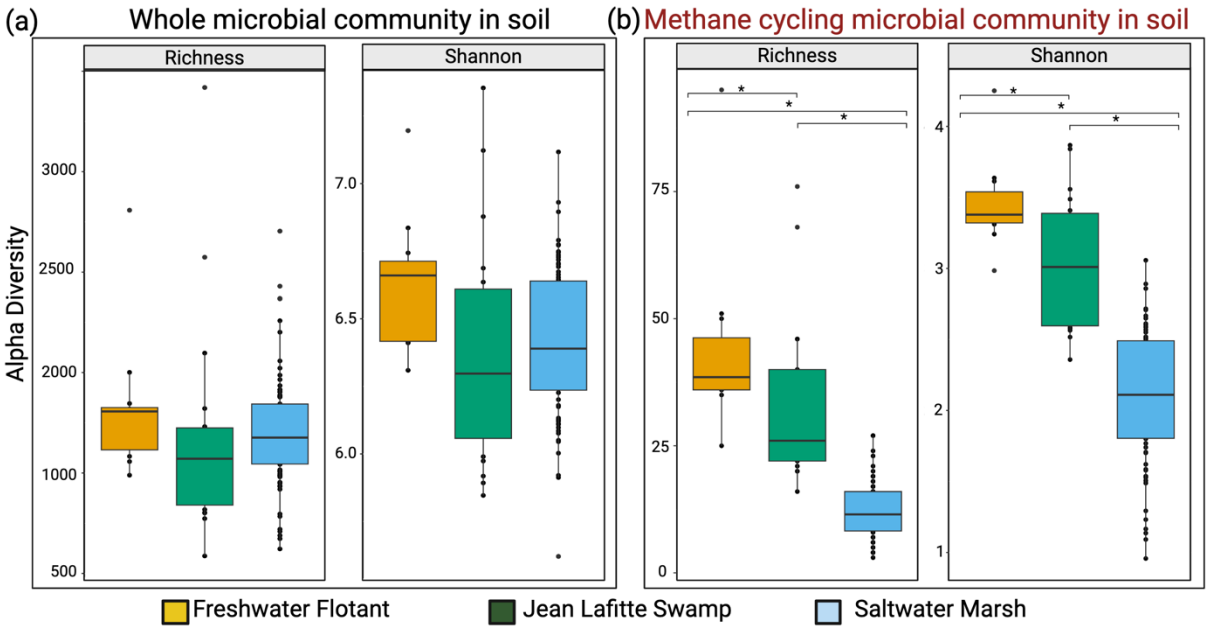

Figure 2.4. Alpha diversity (richness and Shannon Index) of (a) the whole microbial community and (b) methane cycling microbial community in soils of the three wetlands. Boxplots represent the median (horizontal black line inside the box), lower and upper quartiles (25% and 75% of interquartile ranges), ranges (5% and 95%, vertical lines) and outliners (black dots) for each diversity measurement. Brackets are comparing richness and Shannon Index between wetland types (*$p<0.05$).

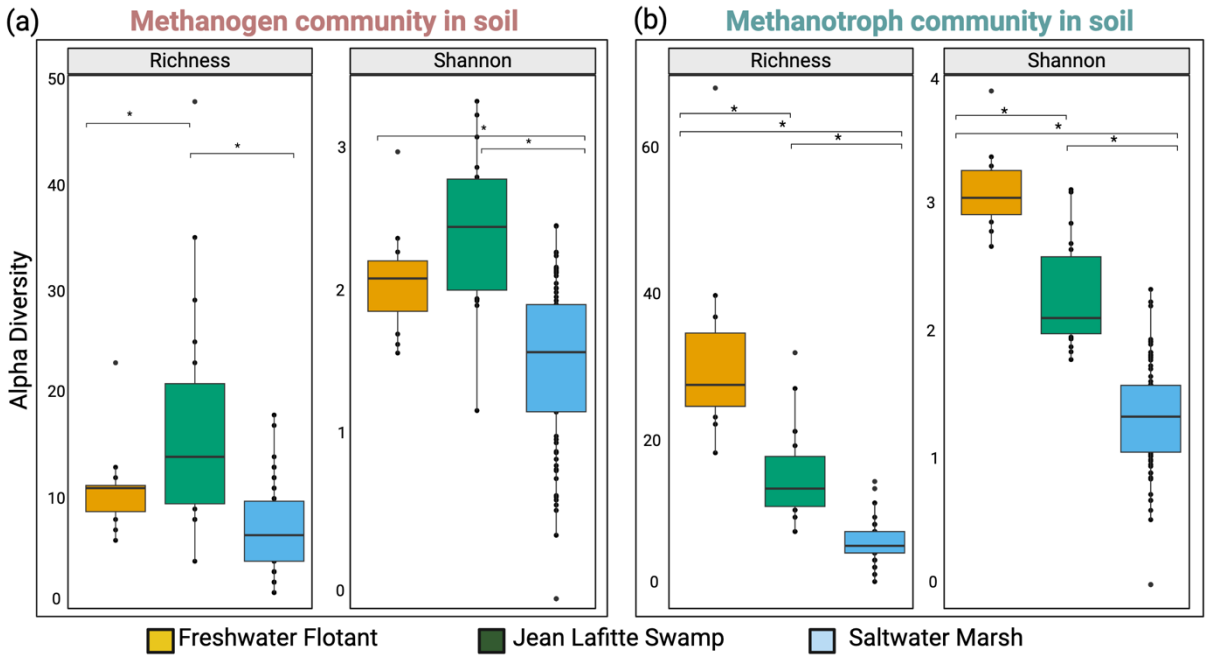

Figure 2.5. Alpha diversity (richness and Shannon Index) of (a) methanogen community and (b) methanotroph community in soils of the three wetlands. Boxplots represent the median (horizontal black line inside the box), lower and upper quartiles (25% and 75% of interquartile ranges), ranges (5% and 95%, vertical lines) and outliners (black dots) for each diversity measurement. Brackets are comparing richness and Shannon Index between wetland types (*$p<0.05$).

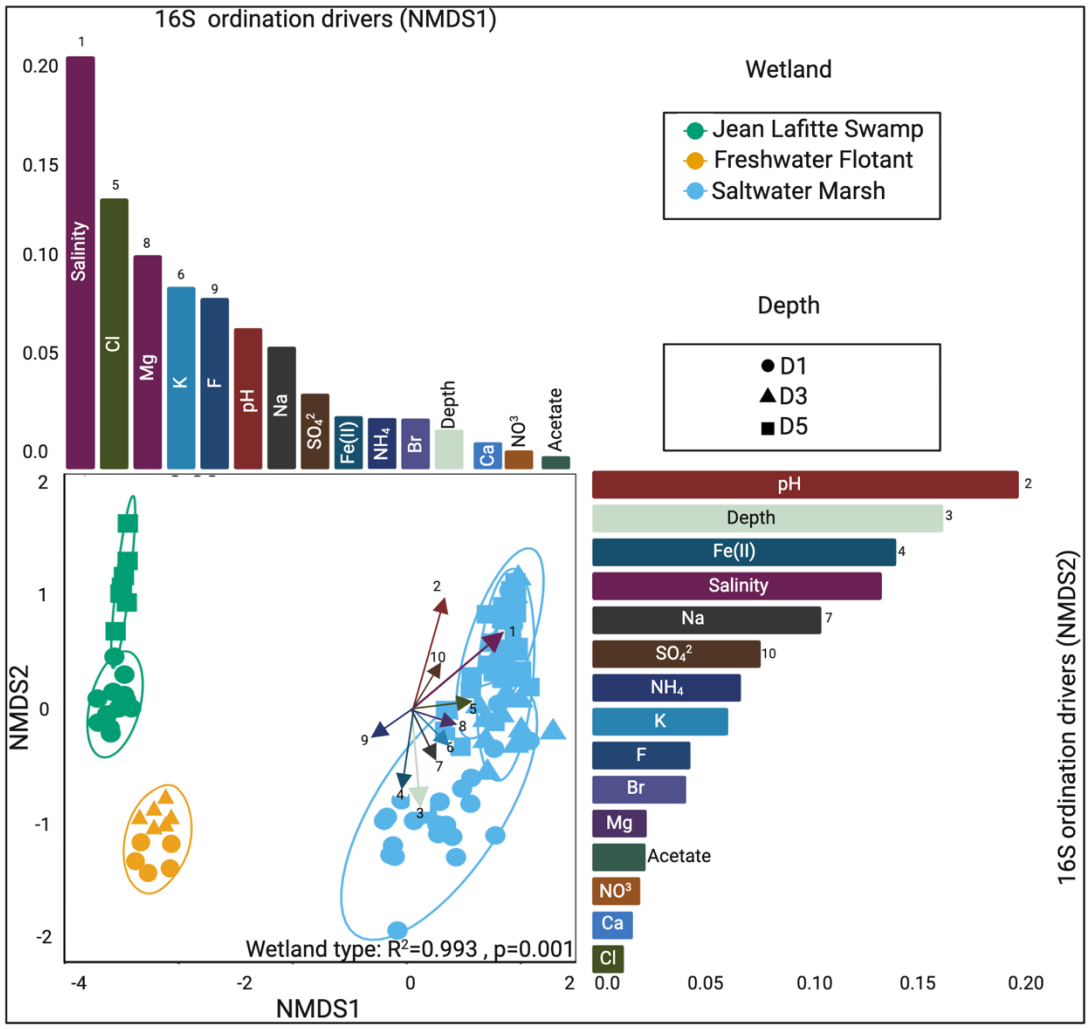

Figure 2.6. Non-metric multidimensional scaling (NMDS) plot of 16S rRNA gene data from whole microbial community in soils of the three wetlands and depth measured with Bray-Curtis dissimilarity index. Bar plots show the biogeochemical compounds driving the ordination with the 10 main biogeochemical compounds numbered for each plot. The factors driving NMDS axis 1 are shown in the bar chart along the top, while those representing the spread along NMDS axis 2 are on the bar chart on the right side of the figure. Geochemical compounds abbreviations are: fluoride (F), iron oxide (Fe(II)), chloride (Cl), nitrate (NO3), sulfate (SO42-), magnesium (Mg), bromide (Br), ammonium (NH4+), sodium (Na), calcium (Ca) and potassium (K).

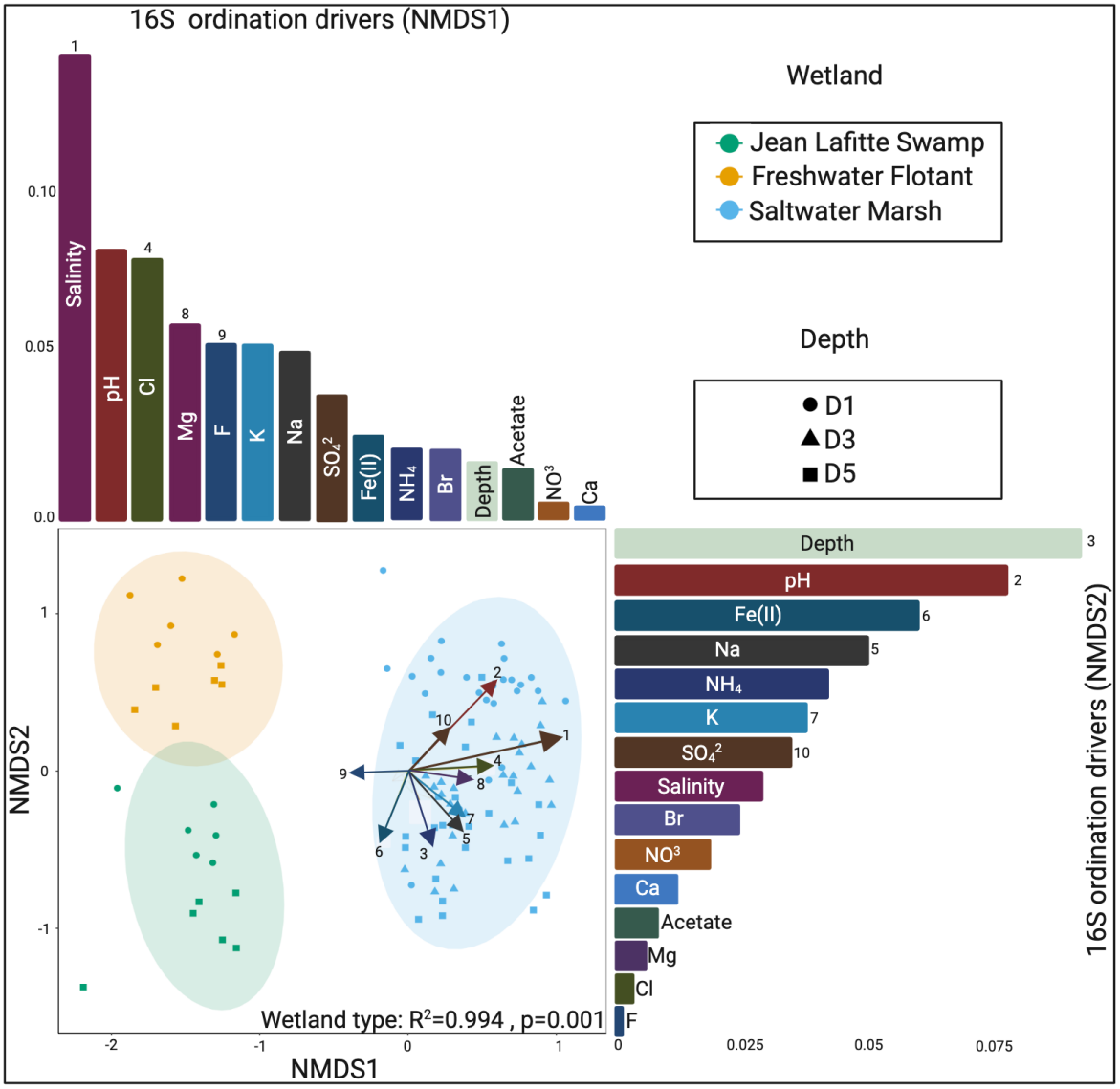

Figure 2.7. Non-metric multidimensional scaling (NMDS) plot of 16S methane cycling microbial communities in soils of three wetlands and depth measured with Bray-Curtis dissimilarity index. The factors driving NMDS axis 1 are shown in the bar chart along the top, while those representing the spread along NMDS axis 2 are on the bar chart on the right side of the figure. The 10 main biogeochemical compounds are numbered for each plot. Geochemical compounds abbreviations are: fluoride (F), iron oxide (Fe(II)), chloride (Cl), nitrate (NO_3), sulfate (SO_4^{2-}), magnesium (Mg), bromide (Br), ammonium (NH_{4+}), sodium (Na), calcium (Ca) and potassium (K).

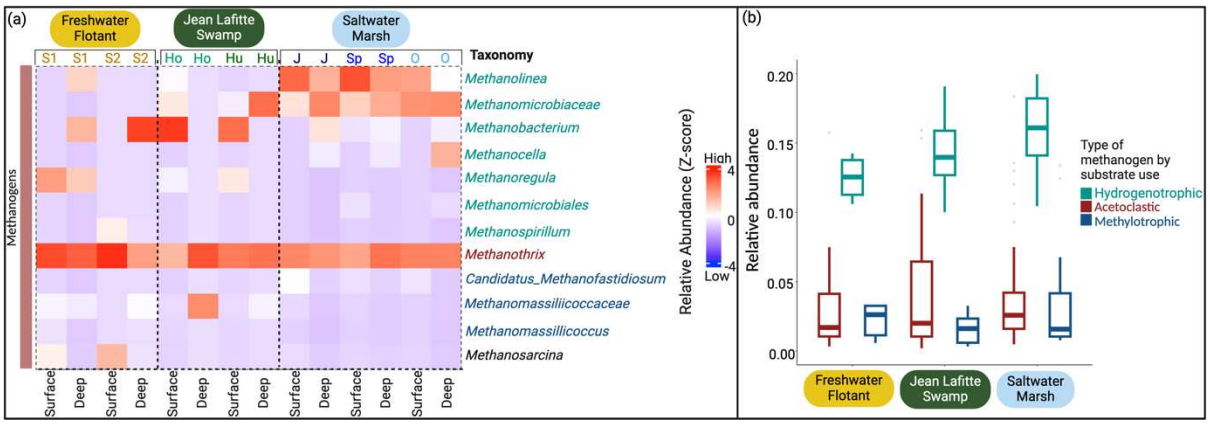

Figure 2.8. Heatmap of methanogen relative abundance grouped by substrate use in soils of the three wetlands with mean data across each ecosite shown by column with the surface and deep proportions noted (a). Relative abundance of methanogens in soil classified by the three predominant substrate functional guilds (hydrogenotrophic, acetoclastic, and methylotrophic) (b). Boxplots represent the median (horizontal black line inside the box), lower and upper quartiles ((25% and 75% of interquartile ranges), ranges (5% and 95%, vertical lines) and outliners (black dots) for each diversity measurement. Brackets are comparing richness and Shannon Index between wetland types (*$p<0.05$). Within each wetland, the proportion of hydrogenotrophic methanogens were enriched significantly from the other functional types. Functional types did not differ along the salinity gradient ($p<0.05$).

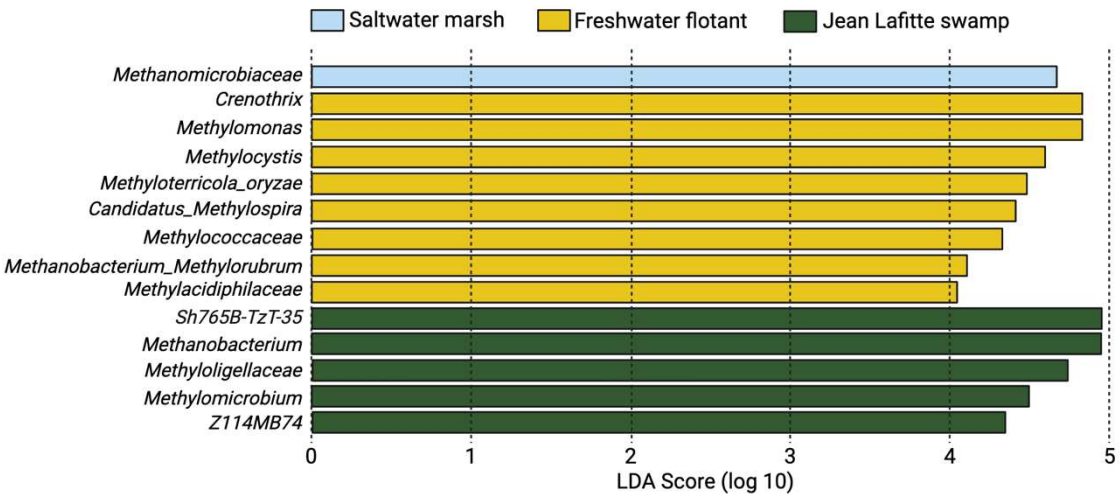

Figure 2.9. Linear discriminant analysis effect size (LEfSe/LDA Effect Size) at the genus level in soils to identify discriminating methanogen and methanotroph taxonomy among three wetlands.

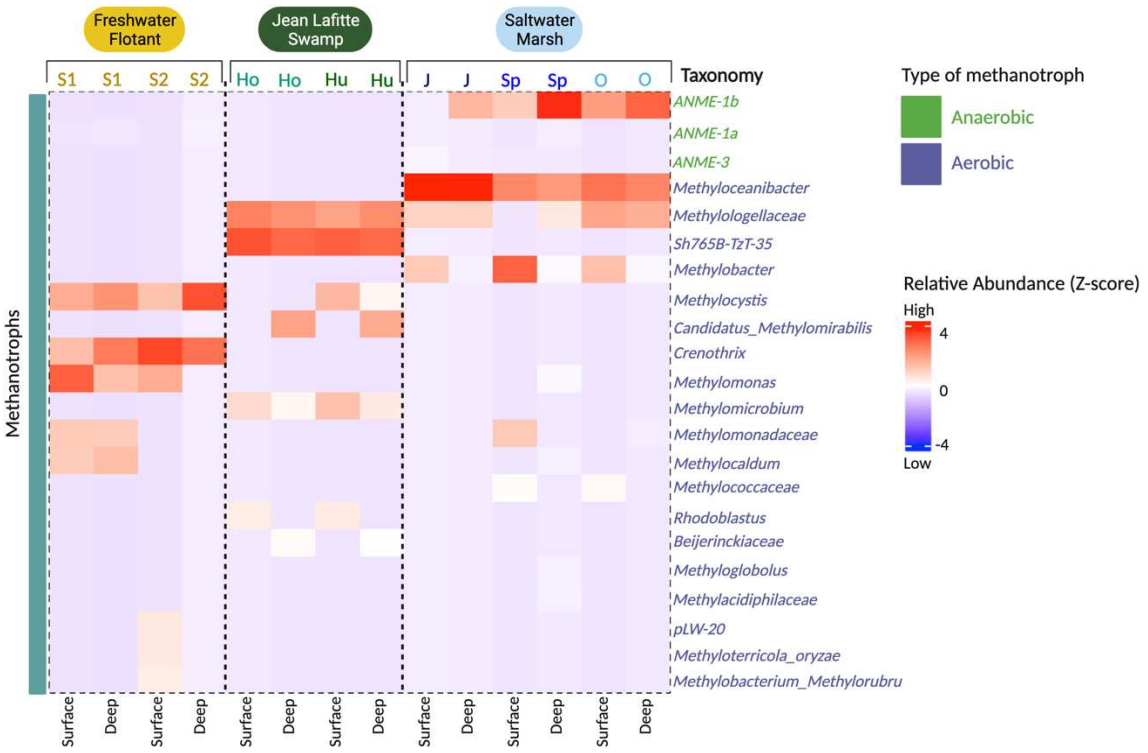

Figure 2.10. Heatmap of methanotroph relative abundance in soils of the three wetlands with mean data across each ecosite shown by column with the surface and deep proportions noted. Relative abundance of methanotrophs in soil is classified by their anaerobic or aerobic characteristic system.

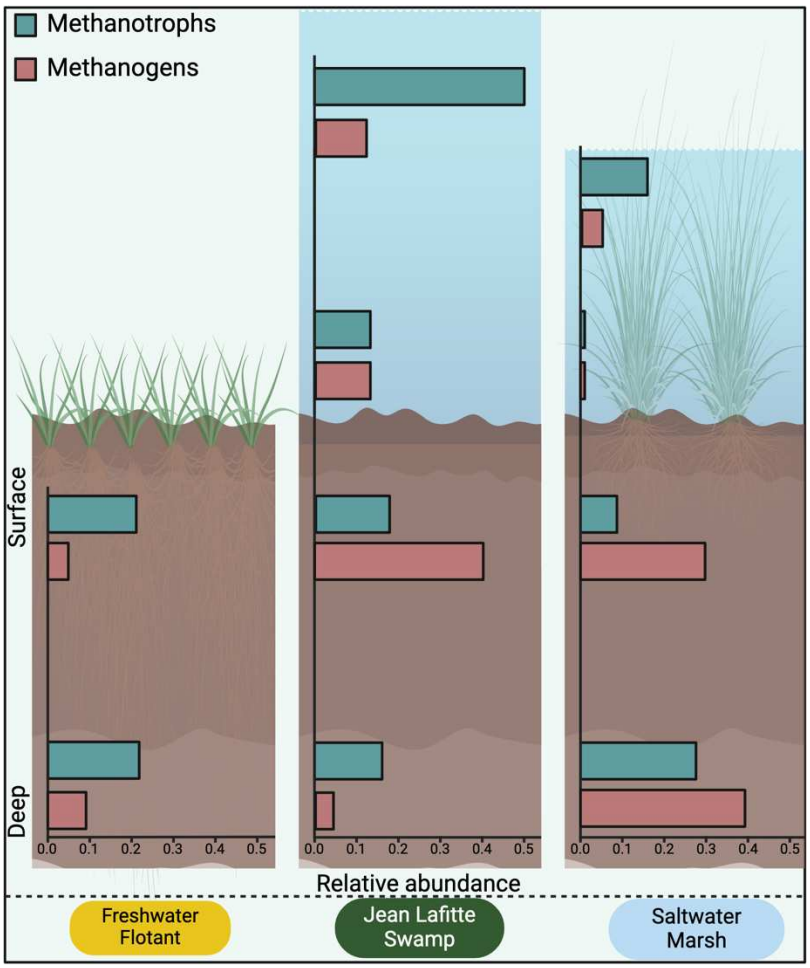

Figure 2.11. Relative abundance of methanogens and methanotrophs in soil and water samples.

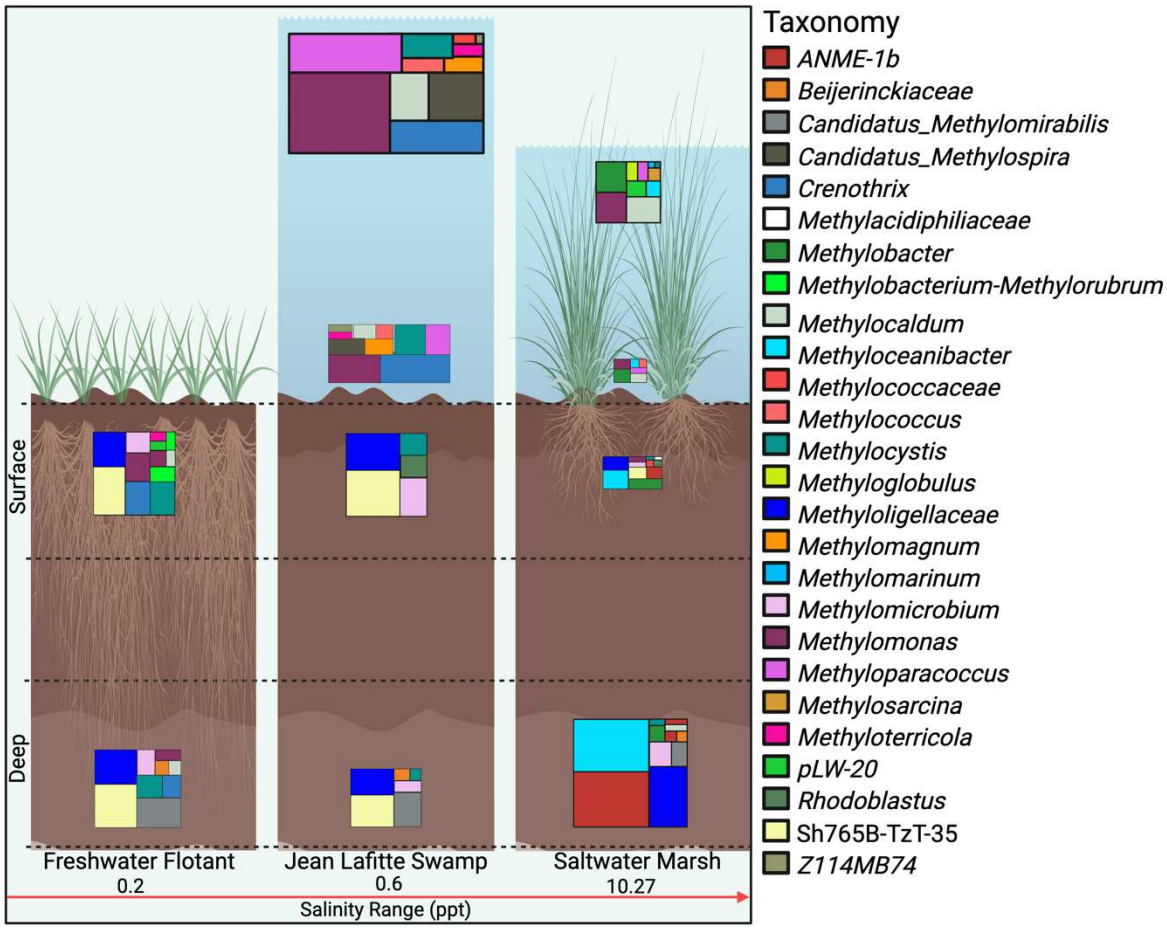

Figure 2.12. Tree map of methanotroph taxa in soil and water samples of three wetlands of Louisiana Barataria Bay, following a salinity gradient. Each block is sized by the proportional relative abundance of methanotrophs by depth and wetland and colored by the proportion of each taxonomy contributing to the total relative abundance of each depth within each wetland.

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CHAPTER 3: CONCLUSION

3.1 Summary

This thesis research elucidates the importance of evaluating environmental characteristics of soils and waters from coastal wetlands, and their relationship with the microbial community abundance and distribution across different wetland types to expand information and create more accurate estimations on methane emission (Chapter 1). With our paired flux, geochemical and microbial data that contributes to the understanding of methane emissions in Gulf Coast wetlands, I showed that the methane cycling microbial community distribution follows the salinity gradient, where the freshwater and swamp wetlands share more similar taxonomy than the saltwater marsh wetland, while hydrogenotrophic methanogen abundances are conserved across wetlands (Chapter 2). I observed a decoupling in methane porewater concentration and methane fluxes across wetlands, which could be explained by the presence of methanotrophic communities in the water column and plant variation in aerenchyma transport mechanisms. Finally, I showed that differences in daily salinity, temperature and vegetation types across wetlands are associated with differences in geochemical differences across sites (Appendix A and Appendix B). These differences could influence the microbial distribution across wetlands. Here, I give future research directions that can help expand our understanding of methane dynamics and improve global climate models predicting greenhouse gases across wetlands (Figure 3.1)

3.2 Future research directions (pairing data)

In this study, I used the 16S rRNA gene sequencing analysis to gather information about the taxonomy and relative abundance of microbes in soil and water samples from coastal

wetlands. Beyond microbial composition and abundance, I need to investigate the activity and metabolism of methanogens and methanotrophs to further understand their contributions to methane cycling. I know that taxonomically identifying microbial genes in the environment is an important tool to characterize the ecosystem (Walters et al., 2016). Finding specific genes that meet the requirements for core community across different wetlands, or finding microbial taxa restricted to a wetland based on their salinity level, for example, gives us information about the potentials for methanogenesis across different wetland types, and here especially for freshwater wetlands affected by sea level rise and salinity intrusion. In addition to this approach, I want to in-depth investigate the microbial functionality and metabolic capacities in these ecosystems (Cowan et al., 2015; Garlapati et al., 2019). Isotopic fractionation of methane can also be used as an indicator of methanogenic pathways and methylotrophic consumption in wetlands and contribute to our understanding of methane dynamics in these systems (Cadieux et al., 2016). With this information, I can better determine differences between microbial communities on a genomic scale, and in the case of coastal wetland sciences, I can explore the metabolic pathways most affected by salinity intrusion (Kumar et al., 2021; Shah et al., 2022).

3.3 Future research directions (beyond 16S rRNA)

In this study, I used the 16S rRNA gene sequencing analysis to gather information about the taxonomy and relative abundance of microbes in soil and water samples from coastal wetlands. Beyond microbial composition and abundance, I need to investigate the activity and metabolism of methanogens and methanotrophs to further understand their contributions to methane cycling. I know that taxonomically identifying microbial genes in the environment is an important tool to characterize the ecosystem (Walters et al., 2016). Finding specific genes that meet the requirements for core community across different wetlands, or finding a taxon restricted

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3.4 Future research directions (scaling to many more coastal wetlands across different climatic regions)

In this work, I show the caution in making rules about methane cycling microbes based on a few representative wetlands. Recent studies reveal the power of scaling microbial and methane data across wetlands for more robust understanding on the biogeography of microorganisms and the factors impacting their abundance (Borton et al., 2013). With this approach, I can identify core communities of relevance across different wetlands and discriminant microbes that characterize specific areas. Scaling wetland research to broader areas that capture different climatic regions will help us better interpret environmental conditions affecting wetland biogeochemistry in a way that can dimmish uncertainties in methane predictions and create more efficient greenhouse gas mitigation plans (Thorslund et al., 2017).

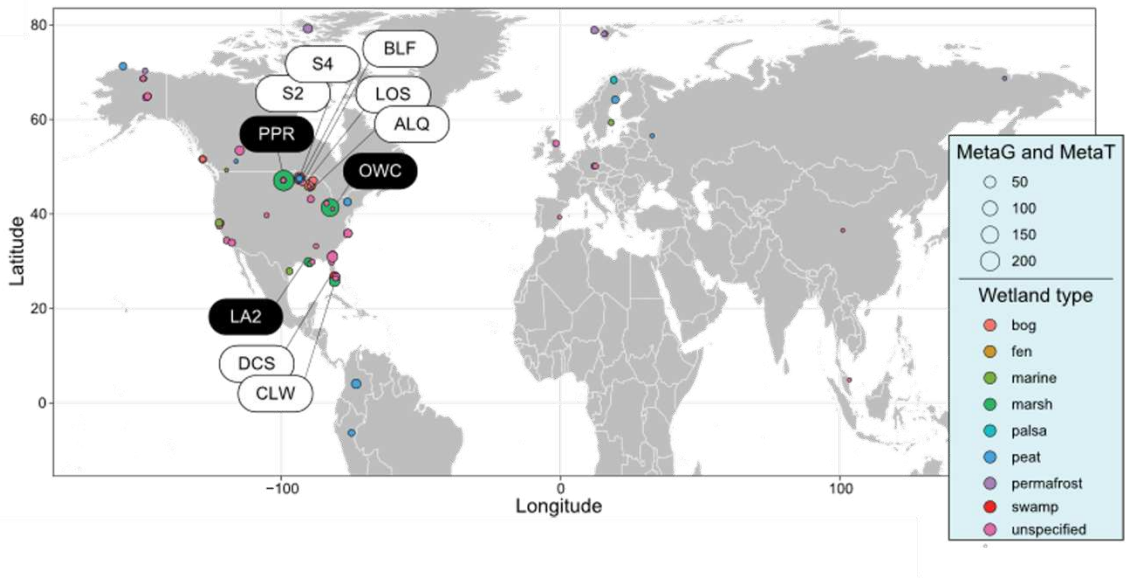

Figure 3.1. Scheme of our sampling to 9 additional wetlands to construct the Multi-omics for Understanding Climate Change (MUCC) database. Points on world map denote wetland sampling locations by our team (10 labeled sites) and others (mined from the Joint Genome Institute Data base). Black labels denote observatory sites, sampled extensively over time points; while white labels denote satellite wetlands, with single time point sampling.

CHAPTER 3: REFERENCES

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APPENDICES

Appendix A: Chapter 2 Supplementary Text

Site description and hydrological characterization

Jean Lafitte National Historical Park and Preserve Swamp (29°80’18″ N, 90°11’02″ W) and AmeriFlux sites US-LA2 Salvador WMA Freshwater Marsh (29°85’87″ N, 90°28’69″ W) and US-LA3 Barataria Bay Saline Marsh (29°49’36’’N, 89°91’53’’W) are in coastal Louisiana. The swamp sites are situated around Lake Salvador, while the freshwater site is located near Lake Cataouatche, and the saltwater sites are beyond Little Lake, in proximity to the waters of the Gulf of Mexico. The Ameriflux sites have Eddy Covariance towers measuring in situ carbon fluxes and were chosen for this reason. The swamp site was selected because it is an intermediate wetland site in terms of salinity and has an interesting microtopography that differs from the other two wetlands, which can help us expand our knowledge of the microbial community contribution to the methane cycle under different environmental conditions.

The hydrological characterization of these wetlands was collected from both public data and in situ measurements. Daily measurements of salinity (ppt) and temperature (°C) for the month of October 2021 were collected from the Coastwide Reference Monitoring System (CRMS) website and reported here for the day of sampling, while mean annual precipitation (mm), mean annual temperature (°C), climate description, and water table elevation (m) along the years were collected from the Ameriflux website (Ward et al., 2023). The water table levels (cm) above the soil sampling locations were measured in situ on the days of sampling with a meter stick and are of ~30 cm on the saltwater marsh and ~65 cm on the swamp (Figure B1, Appendix B).

Table A1. Daily salinity and daily temperature of the three wetlands.

Wetland	Daily salinity (ppt)	Daily temperature (°C)
Freshwater Flotant	0.20	25.58
Jean Lafitte Swamp	0.66	23.15
Saltwater Marsh	10.27	22.50

Table A2. Mean annual precipitation (MAP), mean annual temperature (MAT), climate Koeppen and water table elevation of the Ameriflux freshwater and saltwater wetland sites.

Wetland	MAP (mm)	MAT (°C)	Climate Koeppen	Water table elevation (m)
Freshwater Flotant	1655	20.2	Humid Subtropical	-
Saltwater Marsh	1623	20.9	Humid Subtropical	0.2

Vegetation and topological heterogeneity

According to the International Geosphere Biosphere Programme (IGBP), the vegetation of the Freshwater Flotant and Saltwater Marsh wetlands is classified as permanent wetlands (WET), consisting of large land areas permanently covered by a mixture of water and herbaceous vegetation. The Jean Lafitte Swamp is composed of a hummock and hollow microtopography, which consists of a topological alternation of elevated and flatter soil layers, respectively (Nungesser, 2003). Hummocks and hollows of this swamp are surrounded by Cypress-Tupelo trees (*Taxodium distichum* and *Nyssa aquatica*), and these forests have been responsible for a 17 Mt of total carbon incrementation, which is at risk of loss due to sea level rise and consequent saltwater intrusion affecting the forest density (Edwards et al., 2019).

The freshwater wetland is composed of a highly organic flotant marsh covered by *Sagittaria lancifolia* and *Typha latifolia* (Ward et al., 2023). Before our sampling period in October of 2021, these vegetation coverages were clearly separated, but at the time of sampling Hurricane Ida had interfered in their distribution, which became a mixture of both plant types. Thus, I refer to these sites as "Site 1" and "Site 2". The mesohaline saltwater marsh contains 20-40% organic matter soils and is dominated by two vegetation patches, namely *Juncus roemerianus* and *Spartina alterniflora*, and an unvegetated open water patch (Ward et al., 2023). *Sagittaria*, *Typha*, *Juncus* and *Spartina* plants from both freshwater and saltwater marshes have aerenchyma that highly contributes to methane transport to the atmosphere, and the transportation of gases through the aerenchyma of wetland plants is the most important process on vegetated wetlands (Vroom et al., 2022). When compared to unvegetated soils, it significantly improves our understanding of the methane cycle in coastal wetlands.

Geochemistry

In situ measurements of redox potential were conducted on all three wetlands, while dissolved oxygen (DO) levels were only determined in the saltwater marsh wetland (Figure B2, Appendix B). DO showed a similar dissolved oxygen profile across all 3 sites in the saltwater wetland (Figure B3, Appendix B). The air/water interface DO averaged 8% in *Juncus*, *Spartina* and unvegetated open water, then decreased to 2% when the probe first touched the soil, and reached 0% as the soil depth increased. However, these findings are important to show that our wetland soils have favorable conditions for methanogenesis. Consistent with the measured pore water oxygen concentrations, in the saltwater wetland, redox potential patterns changed within each vegetation type (Figure B3, Appendix B). *Juncus* redox potentials were mostly negative, reaching a minimum of -335 mV and maximum of +52 mV, while *Spartina* redox ranged

between -75 mV and +84 mV, and the unvegetated open water site had a redox potential ranging between -61 mV and +169 mV, with mostly positive values. The redox values for the other two wetlands were mostly positive, ranging between -61 mV and +151 mV in the freshwater flotant and -107 mV and +224 mV in the swamp, although the sampling was not complete in the swamp, as the portable redox-meter broke during measurements. These soil redox measurements agree with other reports that redox potential in wetland soils is normally lower than +300 mV, which favors anaerobic processes like methanogenesis, and sulfate and iron reduction (Mobilian and Craft, 2022).

Cations, anions, pH and Fe (II) analysis were measured on stored samples in the laboratory at CSU upon collection. Unsurprisingly, elements that contribute to salinity, such as chloride and sulfate, were higher in the saltwater marsh samples. I note that acetate was only detected in the vegetated sites of this wetland, which suggests that rhizodeposition could increase above microbial consumption levels (Figure B4, Appendix B). Noting that the acetoclastic *Methanothrix* was a core methanogen across all wetlands, it is interesting to observe its dominance and persistence even in low acetate sites. Consistent with these findings, studies indicate that this methanogen is specialized to grow at low acetate concentrations (Stams et al., 2019).

Calcium, magnesium, potassium, ammonium, nitrate and bromide levels were higher in the saltwater wetland, especially in the unvegetated open water sites, while fluoride was not significantly different across wetlands (Figure B4, Appendix B). The pH levels averaged 7.6 on the freshwater flotant, 6.7 on the swamp, and 7.8 on the saltwater marsh (Figure B5, Appendix B). High pH values are reported in saline coastal wetlands and are explained by the increased cations and anions contents in these systems (Zhu et al., 2020; Zhao et al., 2018; Zhao et al.,

2017). The lower pH on swamp sites could explain the higher Fe (II) contents in those sites, which are more expressive than in the freshwater and saltwater wetlands. Lower pH has been indicated as the main reason for the solubility of iron in wetland soils (Karimian et al., 2018), which agrees with the geochemical results with got from the swamp wetland, especially the higher Fe (II) contents.

Appendix A References

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Appendix B: Chapter 2 Supplementary Figures

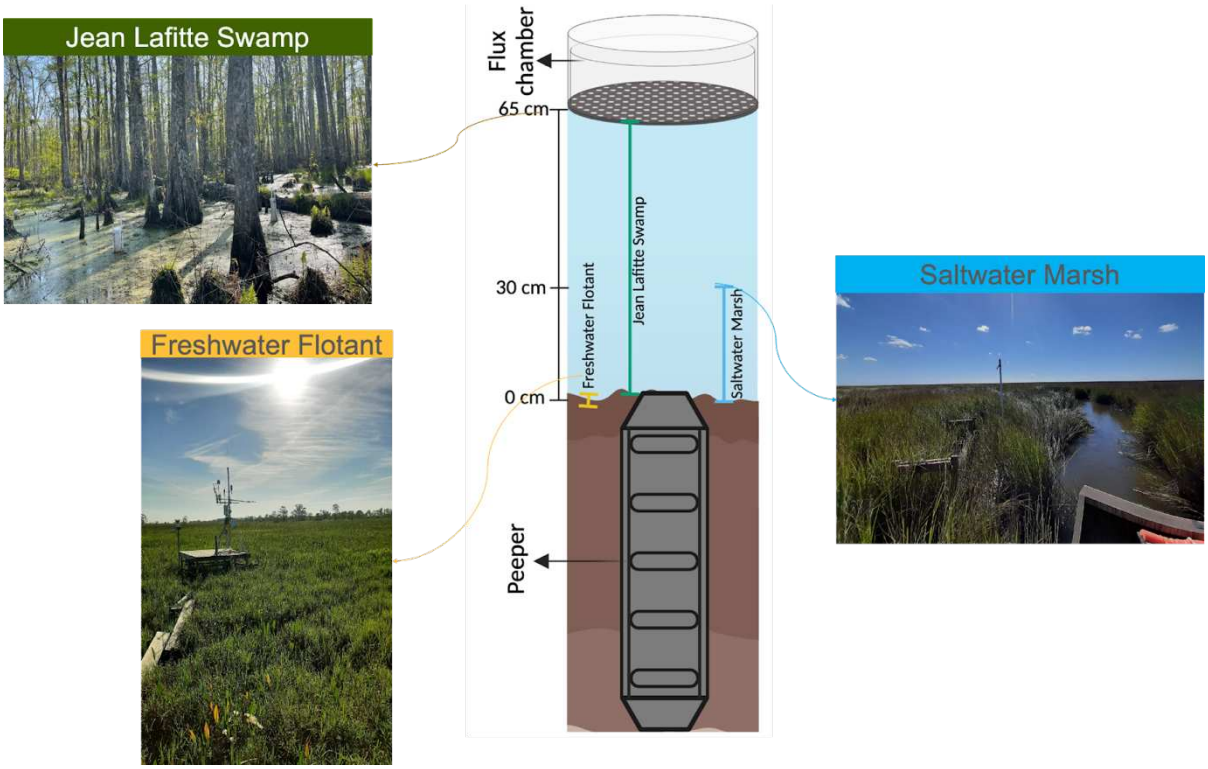

Figure B1. Mean depths (cm) of water table in the three wetlands.

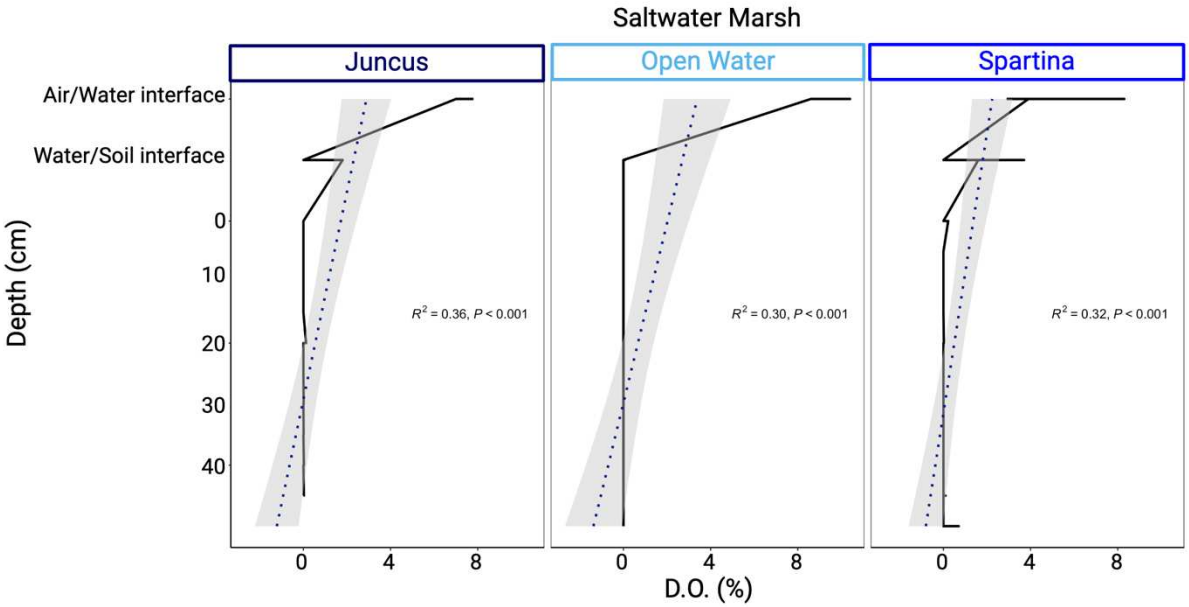

Figure B2. Dissolved oxygen (DO) in water and soil depths of Juncus, unvegetated Open Water and Spartina vegetation covers of the saltwater marsh wetland.

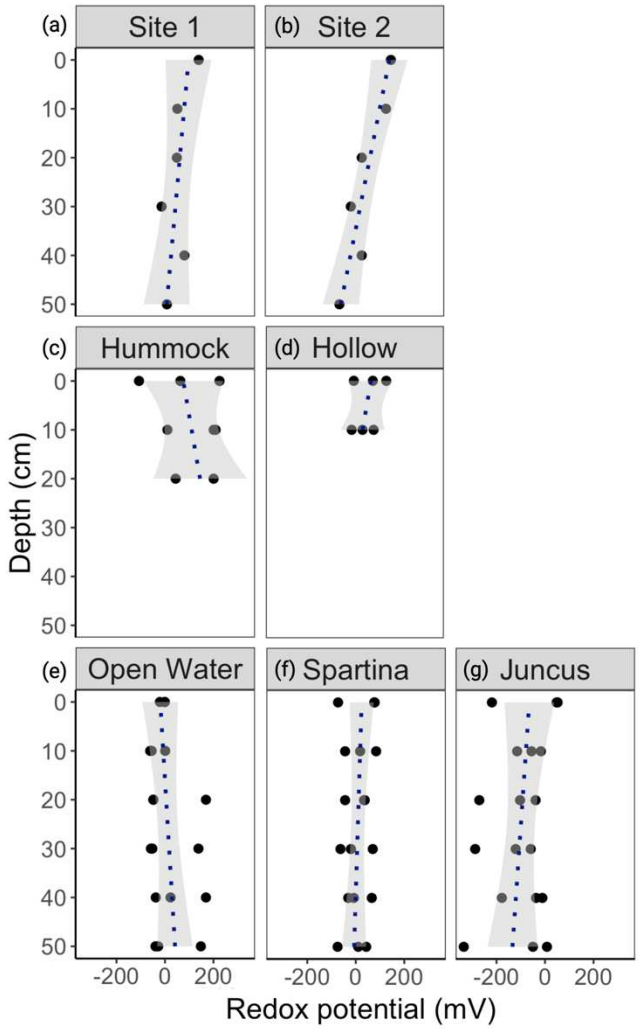

Figure B3. Redox potential (mV) in soils of (a) Site1, (b) Site2, (c) Hummock, (d) Hollow, (e) Open water, (f) Spartina and (g) Juncus vegetation covers of the 3 wetlands.

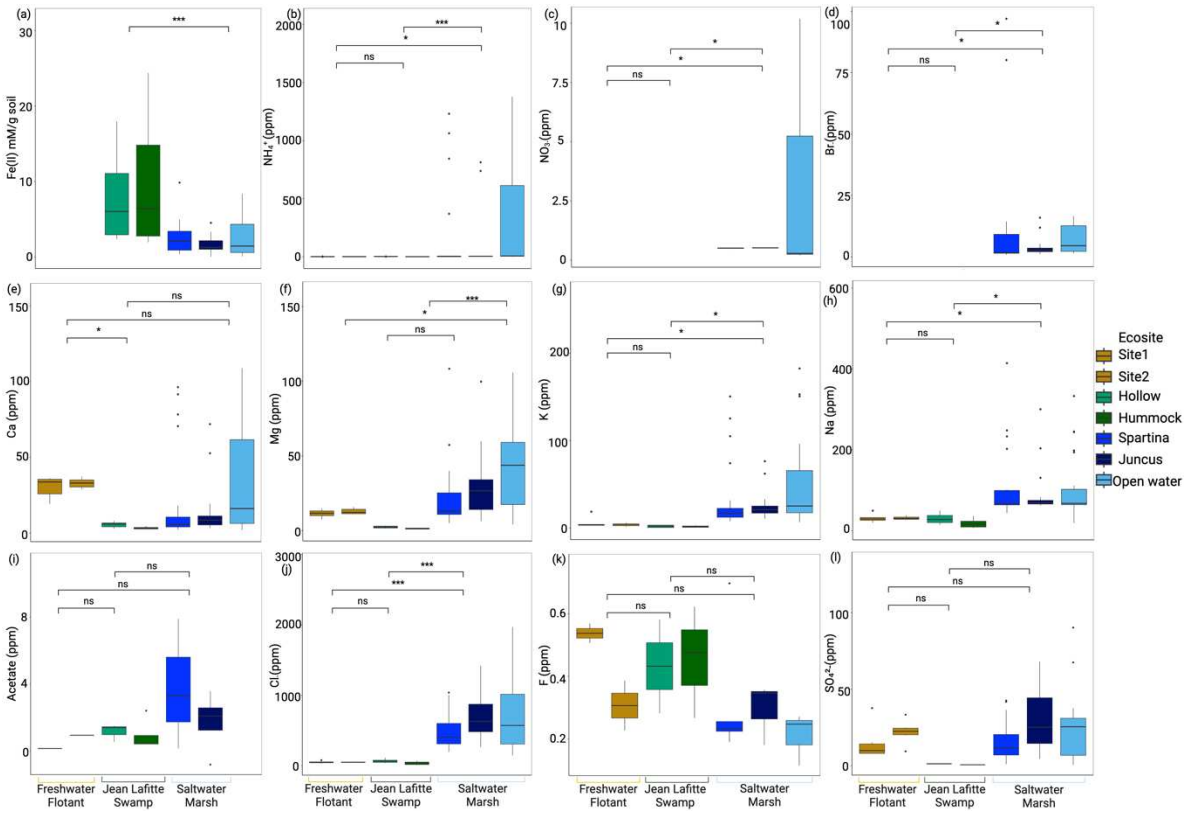

Figure B4. (a) Iron (Fe(II)), (b) ammonium (NH_4), (b) nitrate (NO_3), (b) bromide (Br), (b) acetate, (b) chloride (Cl), (b) fluoride (F), (b) sulfate (SO_4^{2+}), (b) calcium (Ca), (b) magnesium (Mg), (b) potassium (K) and (b) sodium (Na) contents on soils of the three wetlands.

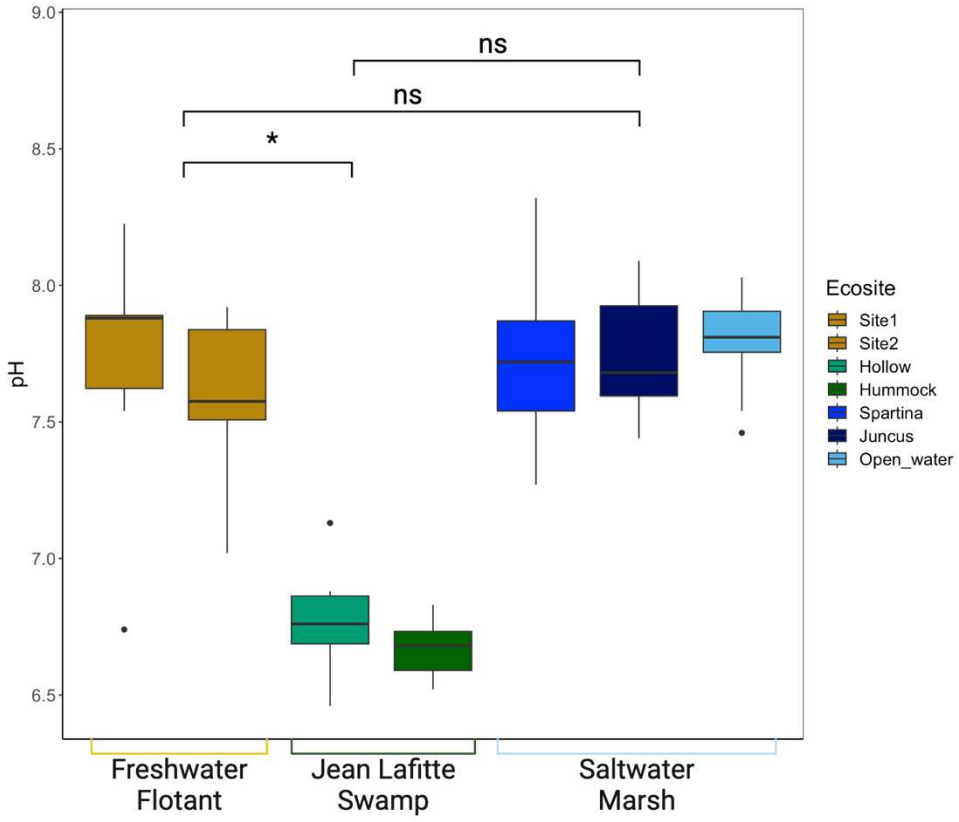

Figure B5. Soil pH of Site1, Site2, Hollow, Hummock, *Spartina*, *Juncus* and unvegetated open water vegetation covers of the 3 wetlands.

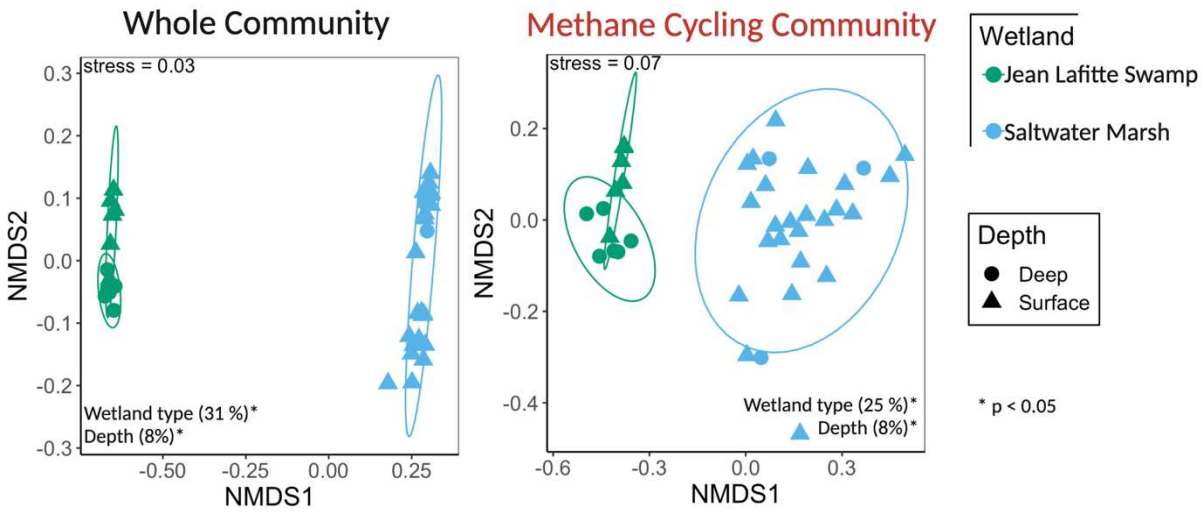

Figure B6. Non-metric multidimensional scaling (NMDS) plot of 16S from whole microbial community and methane cycling community in water samples of Jean Lafitte Swamp and Saltwater Marsh and depth measured with Bray-Curtis dissimilarity index.

Appendix C: Chapter 2 Supplementary Files

Supplementary Data 1. (xlsm) Feature table derived from 16S rRNA gene analyses of whole community.

Supplementary Data 2. (xlsx) Feature table derived from 16S rRNA gene analyses of methane cycling microbial community.

Supplementary Data 3. (xlsx) Paired geochemistry.

Supplementary Data 4. (xlsx) Methane surface flux and methane porewater concentration.