Lawrence Berkeley National Laboratory
LBL Publications

Title
Microbial Ecology and Site Characteristics Underlie Differences in Salinity-Methane Relationships in Coastal Wetlands

Permalink
https://escholarship.org/uc/item/6481s5n4

Journal
Journal of Geophysical Research Biogeosciences, 129(6)

ISSN
2169-8953

Authors
de Mesquita, Clifton P Bueno
Hartman, Wyatt H
Ardón, Marcelo
et al.

Publication Date
2024-06-01

DOI
10.1029/2024jg008133

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, available at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Check for updates

JGR Biogeosciences

RESEARCH ARTICLE
10.1029/2024JG008133

Microbial Ecology and Site Characteristics Underlie Differences in Salinity-Methane Relationships in Coastal Wetlands

Clifton P. Bueno de Mesquita[1], Wyatt H. Hartman[1], Marcelo Ardón[2], Emily S. Bernhardt[3], Scott C. Neubauer[4], Nathaniel B. Weston[5], and Susannah G. Tringe[1,6]

[1]Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, USA, [3]Nicholas School of the Environment, Duke University, Durham, NC, USA, [4]Department of Biology, Virginia Commonwealth University, Richmond, VA, USA, [5]Department of Geography and the Environment, Villanova University, Villanova, PA, USA, [6]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Key Points:

- Salinity-methane relationships are variable across sites
- Relationships between nutrients, alternative electron acceptors, and methane flux were not consistent across sites
- Coastal wetland microbial consortia are site-specific and may respond differently to seawater intrusion, with implications for methane flux

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

S. G. Tringe,
sgtringe@lbl.gov

Citation:

Bueno de Mesquita, C. P., Hartman, W. H., Ardón, M., Bernhardt, E. S., Neubauer, S. C., Weston, N. B., & Tringe, S. G. (2024). Microbial ecology and site characteristics underlie differences in salinity-methane relationships in coastal wetlands. *Journal of Geophysical Research: Biogeosciences*, *129*, e2024JG008133. https://doi.org/10.1029/2024JG008133

Received 11 MAR 2024
Accepted 20 MAY 2024

Author Contributions:

Conceptualization: Clifton P. Bueno de Mesquita, Wyatt H. Hartman, Marcelo Ardón, Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe
Data curation: Clifton P. Bueno de Mesquita, Wyatt H. Hartman, Marcelo Ardón, Scott C. Neubauer, Nathaniel B. Weston
Formal analysis: Clifton P. Bueno de Mesquita
Funding acquisition: Marcelo Ardón, Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe
Investigation: Clifton P. Bueno de Mesquita, Marcelo Ardón, Emily

© 2024. The Author(s).
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Abstract Methane (CH_4) is a potent greenhouse gas emitted by archaea in anaerobic environments such as wetland soils. Tidal freshwater wetlands are predicted to become increasingly saline as sea levels rise due to climate change. Previous work has shown that increases in salinity generally decrease CH_4 emissions, but with considerable variation, including instances where salinization increased CH_4 flux. We measured microbial community composition, biogeochemistry, and CH_4 flux from field samples and lab experiments from four different sites across a wide geographic range. We sought to assess how site differences and microbial ecology affect how CH_4 emissions are influenced by salinization. CH_4 flux was generally, but not always, positively correlated with CO_2 flux, soil carbon, ammonium, phosphate, and pH. Methanogen guilds were positively correlated with CH_4 flux across all sites, while methanotroph guilds were both positively and negatively correlated with CH_4 depending on site. There was mixed support for negative relationships between CH_4 fluxes and concentrations of alternative electron acceptors and abundances of taxa that reduce them. CH_4/salinity relationships ranged from negative, to neutral, to positive and appeared to be influenced by site characteristics such as pH and plant composition, which also likely contributed to site differences in microbial communities. The activity of site-specific microbes that may respond differently to low-level salinity increases is likely an important driver of CH_4/salinity relationships. Our results suggest several factors that make it difficult to generalize CH_4/salinity relationships and highlight the need for paired microbial and flux measurements across a broader range of sites.

Plain Language Summary Sea level rise will lead to increases in salinity in coastal wetlands and estuaries. Salinity is a key variable that controls the amount of greenhouse gases emitted from coastal wetlands, but we do not know if increases in salinity will increase or decrease emissions of the potent greenhouse gas methane, which is produced by microorganisms in wetlands. We examined salinity-methane relationships at four different sites in the United States, and assessed how environmental factors and microorganism communities affected those relationships. We found discrepancies in salinity-methane relationships that are likely driven by site-specific communities of microorganisms that may respond differently to increasing salinity. Relationships between methane emissions and environmental variables were not consistent across sites, highlighting the difficulty of making generalizations about methane emissions from coastal wetlands and how they will respond to seawater intrusion.

1. Introduction

The most recent report by the Intergovernmental Panel on Climate Change stated that increases in well-mixed greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have contributed 1.0–2.0°C of warming to Earth's climate in the past century (IPCC, 2021). After CO_2, CH_4 is the largest contributor, with a global warming potential 84–86 times that of CO_2 over a 20-year timeframe. CH_4 concentrations have increased from 675 ppb in the 1,700 s to 1,866 ppb in 2019, after being stable for most of the previous 800 years (Etheridge et al., 1998; IPCC, 2021). Wetlands are the largest natural source of CH_4; the methanogenic activity of anaerobic archaea in wetland soils contributes approximately 30% of all methane emissions globally (Saunois et al., 2020). It is thus important to understand the drivers of wetland CH_4 emissions, especially given that there

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe
Methodology: Clifton P. Bueno de Mesquita, Wyatt H. Hartman, Marcelo Ardón, Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston
Project administration: Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe
Resources: Marcelo Ardón, Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe
Supervision: Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe
Visualization: Clifton P. Bueno de Mesquita
Writing – original draft: Clifton P. Bueno de Mesquita
Writing – review & editing: Clifton P. Bueno de Mesquita, Wyatt H. Hartman, Marcelo Ardón, Emily S. Bernhardt, Scott C. Neubauer, Nathaniel B. Weston, Susannah G. Tringe

has been much recent discussion of the potential for coastal and estuarine wetlands to sequester vast quantities of carbon in what has been referred to as "blue carbon" (Grimsditch et al., 2013; Kelleway et al., 2020; Mcleod et al., 2011; Nellemann, 2009). However, any increases in carbon (C) storage could potentially be offset by greenhouse gas emissions (Hemes et al., 2018; Rosentreter et al., 2021; Valach et al., 2021), which warrants further study and quantification.

Another topic in need of further research is how minor increases in salinity will affect greenhouse gas emissions, especially given that two principal factors driving coastal and estuarine wetland salinization—drought and sea level rise—are predicted to increase in the future with ongoing climate change (Herbert et al., 2015). Such salinization causes former tidal freshwater wetlands to become oligohaline (>0.5–5 ppt). Globally, sea levels have risen 20 cm since 1901 and are currently (2006–2018) rising at a rate of 3.7 mm per year, as a result of both melting ice and thermal expansion (IPCC, 2021). Droughts have increased in frequency and severity in many parts of the world, which decreases freshwater inputs into estuarine and coastal ecosystems, leading to low-level increases in salinity (Chamberlain et al., 2020; IPCC, 2021). Several other anthropogenic factors such as water management can also contribute to low-level salinization, which, in turn, precipitates ecosystem changes including loss of biodiversity and ecosystem services (Tully et al., 2019).

Salinity is a key environmental variable that affects plant, bacterial, archeal, fungal, and zooplankton community composition and productivity, as well as biogeochemical processes (Crain et al., 2004; Lozupone & Knight, 2007; Mohamed & Martiny, 2011; Weston et al., 2006; Zervoudaki et al., 2009). Only certain organisms in the tree of life have evolved to function at elevated salt concentrations, either by using compatible solutes to keep salt out or by having acidic proteomes and special ion pumps to function with high intracellular salt concentrations (Gunde-Cimerman et al., 2018; Oren, 2013). Effects of salinity on plant communities have been well documented, with drastic observed compositional changes along salinity gradients, including low-level, or oligohaline, salinities (Odum, 1988). High salinities are toxic to plants adapted to freshwater, while plants adapted to saltwater are outcompeted by freshwater-adapted plants in freshwater environments, and are therefore only found in saltwater environments (Crain et al., 2004). Salinity has been found to be a principal variable structuring microbial communities at global, regional, and landscape scales (Dragone et al., 2021; Hartman et al., 2024; Laas et al., 2022; Lozupone & Knight, 2007). At the chemical level, salinity also affects the solubility of dissolved organic carbon and other compounds, as well as the sorption of inorganic ions (Ardón et al., 2013; Neubauer et al., 2013; Weston et al., 2010). While oligohaline salinities are high enough to elicit responses in microbial communities (Hu et al., 2020; Lew et al., 2022), work done across broader salinity gradients including mesohaline (5–18 ppt) and polyhaline (18–30 ppt) areas suggests that the effects of salinization will likely be dependent on the magnitude of salinization (Hartman et al., 2024; Morina & Franklin, 2022; Weston et al., 2014). This may in turn affect the relationship between salinity and methane emissions, which has been shown to be non-linear (Hartman et al., 2024).

Theory predicts that CH_4 emissions will decrease with increased seawater influence as the concurrent increase in sulfate from seawater will promote sulfate-reducing organisms that can outcompete methanogens for shared resources, such as acetate and hydrogen (Achtnich et al., 1995; Kristjansson & Schönheit, 1983; Lovley & Klug, 1983; Lovley et al., 1982; Schönheit et al., 1982). Such a decrease, in fact a log-linear decrease in CH_4 emissions with salinity (including oligohaline salinities up to seawater salinities), is indeed what has been found in several studies and meta-analyses (Al-Haj & Fulweiler, 2020; Bartlett et al., 1987; Luo et al., 2019; Poffenbarger et al., 2011). However, a summary of laboratory microcosm experiments that tested salinity-methane relationships reported 8 negative relationships, 2 *positive* relationships, and 1 neutral relationship (Ardón et al., 2018). Similar variation has been observed for CO_2 emissions (Luo et al., 2019). Such variation in relationships has been attributed in part to hydrological setting (Ardón et al., 2018; Helton et al., 2019) and soil characteristics (Ury et al., 2022), but the roles of site context/history, including legacy effects of agriculture and fertilizer use (Ardón et al., 2017), as well as microbial ecology—relations of microbes to one another and to their physical surroundings—are two other main factors that could contribute to such discrepancies and are in need of further research. Wetlands with a history of agriculture use, and consequently fertilizer application, are characterized by what are known as biogeochemical legacies, including altered carbon and nutrient pools, such as legacy nitrogen and phosphorus (P) that can remain in soils for decades after the cessation of inputs (Ardón et al., 2017). Other important site legacies include the history of inundation and prior exposure to salinity (Hopple et al., 2022).

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CH_4 cycling is driven by the activity of, and interactions among, multiple distinct functional groups of microorganisms, including complex carbon degraders, fermenters, sulfate reducers, iron reducers, ammonia oxidizers, denitrifiers, methanogens, and methanotrophs (Bodelier & Steenbergh, 2014; Conrad, 2020; Lovley, 1991). These groups encompass functionally and taxonomically diverse organisms. Complex carbon degraders and fermenters are crucial players in the decomposition process, breaking down larger, more recalcitrant carbon compounds such as lignin and cellulose into fatty acids and eventually acetate, H_2 and CO_2 that fuel methanogenesis (Conrad, 2020). While sulfate reducers can compete with methanogens, they can also be syntrophic with methanogens, as certain taxa produce acetate and hydrogen which would then fuel methanogenesis from those substrates. Methanogens can perform one of four different methanogenesis pathways, including acetoclastic (CH_4 produced from acetate), hydrogenotrophic (CH_4 produced from hydrogen and CO_2 or other electron acceptors such as carbon monoxide or alcohols), methyl-dismutation (i.e., methylotrophic, CH_4 produced from methylated compounds), and methyl-reduction (CH_4 produced from methylated compounds and hydrogen) (Bueno de Mesquita et al., 2023; Kurth et al., 2020). Most methyl-dismutation pathways are likely not affected by competition with sulfate reducers (Maltby et al., 2016; Oremland & Polcin, 1982; Oremland et al., 1982; Xu et al., 2021). Methanotrophs include anaerobic archaea and both anaerobic and aerobic bacteria (Conrad, 2007; Guerrero-Cruz et al., 2021), the latter of which have been divided into separate types based on taxonomy and physiology (here we use types I, II, IIa) (Bodelier, 2011; Hanson & Hanson, 1996; Knief, 2015). Furthermore, prior work has suggested key interactions between the methane and nitrogen cycles, and among the taxa involved, particularly methanotrophs, ammonia oxidizers (AO) and nitrite oxidizing bacteria (NOB) (Bodelier & Steenbergh, 2014). Nitrogen can stimulate CH_4 oxidation in N limited conditions, while excess ammonia and nitrite can inhibit CH_4 oxidation (Alam & Jia, 2012; Cai et al., 2007; Dunfield & Knowles, 1995). Such diversity of microorganisms may lead to contrasting responses or a lack of response to low salinity, depending on the direct effects of salinity on these taxa, the indirect effects of other environmental variables on these taxa, the effects of interactions among these taxa, and the overall functional redundancy of the microbial community (Bodelier, 2011; Morrissey, Berrier, et al., 2014; Nyerges & Stein, 2009).

In this study we combine microbial and biogeochemical data from field and laboratory experiments in four different sites to assess how salinization affects microbial communities and biogeochemistry, including greenhouse gas fluxes. These data encompass tidal freshwater marshes, oligohaline wetlands, a freshwater forested wetland, control microcosms and microcosms amended with artificial seawater (ASW), field plots amended with artificial seawater, and tidal freshwater soils transplanted to an oligohaline wetland. With respect to microbial ecology, we hypothesized that (H1) low salinity would reduce alpha-diversity within each site due to the direct effects of NaCl on freshwater-adapted taxa, and (H2) broad site geographic, hydrologic, plant, and biogeochemical differences would lead to significant differences in microbial beta-diversity across the four geographic locations due to the effects of those variables on microorganisms. With respect to relationships with CH_4 flux, we hypothesized that (H3) a greater relative abundance of methanogens would fuel more methanogenesis and a lower relative abundance of methanotrophs would result in more CH_4 emitted to the atmosphere, (H4) increased availability of alternative electron acceptors would suppress methanogenesis due to competition, and (H5) greater overall decomposition rates would fuel more methanogenesis by providing methanogenic substrates. To test H5 we used correlates of decomposition, including CO_2 flux, organic carbon, ammonium, phosphate, pH, and relative abundance of hypothesized complex C degraders (Actinobacteriota, Firmicutes). While we did not directly measure decomposition rates, the literature shows that decomposition rates are positively correlated with carbon availability (Luo et al., 2015), as well as nutrient availability, including nitrogen and phosphorus (Barantal et al., 2012; Yan et al., 2020). Nutrient availability could also increase plant growth, but this in turn could increase carbon inputs and fuel increased decomposition. Long-term nutrient addition experiments have also been shown to increase CH_4 fluxes (Juutinen et al., 2018). Regardless of potential pH effects on methanogens, research has shown that overall decomposition rates are higher at more neutral pH compared to acidic pH (Walse et al., 1998). Firmicutes and Actinobacteriota were shown to harbor complex carbon degradation genes in wetlands (Hartman et al., 2024), so we also include them in this prediction.

2. Methods

We synthesized data from five research projects from four separate sites, chosen because they included measurements of methane fluxes in freshwater and oligohaline conditions, either due to natural variation or to field or lab manipulation, and had retained soil samples adequate for DNA extraction and sequencing for microbial

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

community characterization, which had not been previously done at three of the four sites. The projects include field sampling across a natural salinity gradient, field manipulations of salinity with either seawater addition or transplanting, and laboratory microcosm incubation experiments in which soils were brought back to the laboratory to receive artificial seawater (ASW) additions for at least 12 weeks. Broadly, the four sites are the San Francisco Bay Estuary in California, USA ("SF") (Hartman et al., 2024), the Delaware River Estuary in New Jersey, USA ("DE") (Weston et al., 2014), the Alligator River Estuary in North Carolina, USA ("Alli") (Ardón et al., 2010, 2013), and the Waccamaw River Estuary in South Carolina, USA ("Wacc") (Neubauer, 2013) (Table 1, Figure S1 in Supporting Information S1). These estuaries contain vast expanses of wetlands; for example, there are ∼59,000 ha of wetlands in the San Francisco Estuary and ∼445,000 ha and of wetlands in the Delaware Estuary. From all of the potential data available, we selected only samples with freshwater (<1 ppt) or oligohaline (1–5 ppt) salinity classes, as this is the most important salinity class to understand regarding seawater intrusion into freshwater wetlands and tidal marshes (Table 1). All soil samples were continuously covered with water (execpt Delaware lab) and come from similar depth classes within the 0–5 and 5–15 cm range. All soil samples were exposed to increased salinity for at least 12 weeks, but the length of exposure varied among studies, including decades (San Francisco), 3 years (Waccamaw), 1 year (Delaware field), and 12 weeks (Delaware lab, Alligator lab). CH_4 flux was measured at all sites; however, the surface area, volume, and depth of the sample upon which measurements were taken were different at each site, and some sites used a gas chromatograph while a portable analyzer was used at San Francisco (Table 1). Thus, methane flux data are comparable among treatments in the same site but are not directly comparable among the sites (Table 1). Additionally, CO_2 flux was measured at San Francisco, Waccamaw, and Alligator, and N_2O flux was also measured at Delaware and Alligator. Suites of other soil and porewater variables were measured, and this varied among the sites and experiments as described below. Variables measured at only one site were included in the analysis if similar variables were measured at other sites (e.g., soil organic matter, dissolved organic carbon).

2.1. Sampling and Measurements

In San Francisco, soils were collected from 2 freshwater and 2 oligohaline wetland complexes in the delta formed by the Sacramento and San Joaquin rivers as they empty into the San Francisco Bay. Each salinity class included one unaltered reference wetland and one wetland restored from agricultural use as either cropland or pasture. At each wetland, three cores were taken with a split core auger with an airtight plastic sleeve with a 5 cm diameter and 15 cm depth. Each core in the plastic sleeve was immediately capped on both ends to maintain an anaerobic environment. The core was then placed into a 2 L Mason jar and the top cap was removed to quantify CH_4 and CO_2 flux over the course of 5 min on an ultraportable greenhouse gas analyzer (Los Gatos Research, San Jose, CA, USA). An additional core adjacent to each initial core was taken, split into the 0–5 cm depth segment and 5–15 cm depth segment, and aliquoted for DNA sequencing (stored at −20°C) and biogeochemical analyses of soil and porewater at the UC Davis Analytical Laboratory. Measured variables included: porewater dissolved organic carbon (DOC) and pH, soil C, nitrogen (N), C:N, ammonium (NH_4^+), and Olsen phosphate (PO_4^{3-}), and both porewater and soil nitrate (NO_3^-), sulfate (SO_4^{2-}), iron (Fe), and manganese (Mn). Samples from San Francisco are all unmanipulated field samples (n = 72).

At Waccamaw, a field experiment was established in the 0.9 ha Brookgreen Gardens tidal freshwater marsh (33° 31.50′N, 79°5.51′W), adjacent to Springfield Creek, a tidal tributary of the Waccamaw River (Neubauer, 2013). The site is flooded 10–30 cm during most high tides. Five replicate plots, at least 3 m apart from each other, received 40 L of either freshwater or seawater additions every 3–4 days over the course of 3 years. Seawater additions were from salt marsh tidal creek water from the flow-through seawater system at the Baruch Marine Field Laboratory (BMFL) that was diluted with freshwater to a salinity of 10.2 before being added to the marsh. Freshwater additions were from a 180-m-deep groundwater well at the BMFL (salinity = 0.5 ± 0.04). Plot-scale exchanges of CO_2 and CH_4 were measured with large, transparent, temperature-controlled chambers 0.37 m^2 × 1.22 m height. Air within each chamber was stirred with four fans while pumps circulated air between the chambers and a LI-COR LI7000 CO_2/H_2O analyzer (LI-COR Biosciences, Lincoln, NE, USA). To determine CH_4 fluxes, air samples were collected from each chamber roughly every 5–7 min, stored in gas tight Hungate tubes, and analyzed for CH_4 concentration in the laboratory using a flame ionization detector on a Shimadzu GC14A gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA). Soils were collected at the end of the experiment by collecting one core of 6.4 cm diameter and 10 cm depth from each plot. Other measured

Journal of Geophysical Research: Biogeosciences 10.1029/2024JG008133

Table 1
Summary of the Different Sources of Data Combined in This Paper

Site	Setting	Type	Manip.	Type	Salinity	pH	Plants	Lat.	Long.	History	Depth	GHG	GHG n	DNA n	Ref.
SF	Field	Obs.	None								0–5 cm, 5–15 cm	Core-level, 5 cm diam., 15 cm deep, 2 L chamber, 5 min	36	72	Hartman et al. (2024)
-Sandmound				Tidal FW	0.6	6.1	Tule, cattail, Three-square	38.001	−121.624	Urban/ag. runoff			7	14	
-WestPond				Tidal OH	0.4	6.1	Tule, cattail	38.107	−121.648	Urban/ag. runoff			6	12	
-Mayberry				Tidal OH	2.9	6.8	Tule, cattail	38.05	−121.765	Restored from ag.			18	36	
-Browns				Tidal FW	3.5	6.0	Tule, Three-square	38.046	−121.866	Restored from ag.			6	12	
Waccamaw	Field	Exp.	+SW	Tidal FW	0.1	6.6	Giant cutgrass, groundnut, water hemlock, marsh pennywort, goldenclub, arrow arum, pickerelweed, hastate tearthumb, sagittate tearthumb	33.525	−79.0919	Restored from ag.	0–4 cm, 10–14 cm	Plot-level, 0.37 m^2 area, 1.22 m high, 5–7 min	15	30	Neubauer et al. (2013)
Delaware	Field	Exp.	Transplant								0–4 cm, 10–14 cm	Plot-level, 0.372 m^2, 0.4 m deep, 0.1 m high, 20 min	4	8	NA
-Rancocas				Tidal FW	0.1	NM	Green arrow arum, Spanish needles, Water hemp, dotted smartweed, sagittate tearthumb	39.982	−74.8343	Urban/ag. runoff			TP source	TP source	
-Raccoon				Tidal FW	0.1	NM	Green arrow arum, Spanish needles, Water hemp, dotted smartweed, sagittate tearthumb	39.79	−75.3573	Urban/ag. runoff			2	4	
-Salem				Tidal OH	1.8	NM	Wild rice	39.624	−75.452	Urban/ag. runoff			2	4	
Delaware	Lab	Lab	+ASW	Tidal FW	0.2	7.5	Green arrow arum, pickerelweed, yellow pond lily	39.859	−75.1731	Urban/ag. runoff	0–4 cm, 10–14 cm	Core-level, 10 cm diam., 25 cm deep, 1.2 L chamber, 1 hr	4	8	Weston et al. (2011)
Alligator	Lab	Lab	+ASW	Forested FW	0.1	3.9	Atlantic white cedar	35.906	−76.1569	Restored from ag.	0–5 cm, 5–15 cm	Core-level, 2.5 cm diam., 30 cm deep, 1 hr	8	15	Ardón et al. (2013, 2018)

Note. SW = seawater, ASW = artificial seawater, FW = freshwater, OH = oligohaline, TP = transplant, GHG = greenhouse gas. Scientific names of the plant species are: Tule = *Schoenoplectus acutus*, Cattail = *Typha latifolia*, Three-square = *Schoenoplectus americanus*, Giant cutgrass = *Zizaniopsis miliacea*, groundnut = *Apios americana*, Water hemlock = *Cicuta maculata*, Marsh pennywort = *Hydrocotyle umbellata*, Goldenclub = *Orontium aquaticum*, arrow arum = *Peltandra virginica*, Pickerelweed = *Pontederia cordata*, hastate tearthumb = *Polygonum arifolium*, sagittate tearthumb = *Polygonum sagittatum*, dotted smartweed = *Polygonum punctatum*, Green arrow arum = *Peltandra virginica*, Spanish needles = *Bidens* sp., Water hemp = *Amaranthus rudis*, Wild rice = *Zizania aquatica*, Yellow pond lily = *Nuphar lutea*. Salinity is mean ppt, pH is mean pH. NM = not measured.

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

variables included soil organic matter (SOM), C, N, and C:N, and soil oxygen demand (SOD). Samples from Waccamaw are all manipulated field samples (n = 30).

At Delaware, data come from two separate projects—a field transplant experiment and a laboratory incubation experiment. For the Delaware transplant experiment, large (29 cm diameter and approximately 30 cm depth) intact cores of tidal freshwater marsh (Rancocas Creek) soil and vegetation were transplanted into an oligohaline wetland (Salem) and a separate tidal freshwater marsh (Raccoon Creek) to serve as a control. Tidal freshwater marsh plant communities were dominated by *Peltandra virginica* and species of *Bidens*, *Amaranthus*, and *Polygonum*, while the oligohaline site was dominated by *Zizania aquatica* (wild rice). Transplants were done at water level as well as 40 cm below water level, but only the 40 cm flooded samples are included here, to more closely match the Delaware laboratory data (see below) and the Waccamaw field experiment (see above). Soil for DNA sequencing was taken from 0 to 4 cm and 10–14 cm depth. Greenhouse gas measurements were taken by placing covered (dark) acrylic chambers of 43 L volume over the soil cores. Carbon dioxide production inside the chamber was measured with a PPSystems infrared gas analyzer for several minutes. Methane exchange was measured by taking gas samples at 0, 10, and 20 min with a 60 mL syringe, injecting into 20 mL evacuated vials, and measuring CH_4 on an Agilent 6890N gas chromatograph with a flame ionization detector. Other measured variables here included porewater NH_4^+, PO_4^{3-}, SO_4^{2-}, and Fe^{2+}.

For the Delaware laboratory microcosm incubation experiment, soils from the Woodbury Creek tidal freshwater marsh were collected to a depth of 25 cm with 10 cm diameter polyvinyl chloride tubes and sealed with gas- and water-tight caps (Weston et al., 2011). Holes were drilled in the core barrel just above the soil surface, and then cores were placed into separate 100 L tidal tanks in the dark at 20°C. The tidal tanks simulate the tidal cycle with 6 hr of air exposure followed by 6 hr of inundation. Tanks were filled with artificial freshwater that mimicked the freshwater chemistry of the Delaware River. After a 2-week pre-incubation period, water in one tank was replaced with artificial seawater with a salinity of 4.95 ppt. Both tanks were changed at least once weekly after the initial 2-week period. Cores were incubated for 12 weeks after which the 0–4 and 10–14 cm depth portions were sampled and stored at −20°C for DNA sequencing, with an aliquot sent for porewater and soil biogeochemical measurements which included Cl^-, SO_4^{2-}, PO_4^{3-}, DOC, NO_x, and acetate. Gas flux was measured by fitting a 1.2 L gas-tight cap onto the core. An initial headspace sample (3 mL) for CH_4 analysis was obtained with a gas-tight syringe; then final CH_4 samples were obtained after approximately 1 hr. CH_4 samples were analyzed immediately by flame ionization detection gas chromatography (Agilent 6890 N with Porapak Q column). The wetlands that were sampled in both experiments have no known prior history of agricultural use but are affected by runoff from surrounding agricultural and urban areas. Sample sizes from Delaware are n = 8 for the transplant experiment and n = 8 for the laboratory experiment (Table 1).

At Alligator, soils were collected from the Timberlake Observatory for Wetland Restoration, a forested freshwater wetland restored in 2004 from prior use as a corn field that is now used as a research site. The site had not experienced saltwater incursion for at least 20 years prior to sampling. The site where the soils were collected is characterized by Eutric Histosol soils and Atlantic white cedar vegetation (Ardón et al., 2013). A laboratory incubation was started with intact soil cores 2.5 cm in diameter and 30 cm deep. Alligator microcosms received either deionized freshwater (control), artificial seawater, artificial seawater without sulfate, or sulfate. In this analysis we only included the controls and artificial seawater additions, as in the Delaware experiment. To measure CO_2, CH_4, and N_2O, cores were fitted with a gas tight lid with a Swagelok brass sampling port with a rubber septum (0.6 cm). Headspace gas samples were collected immediately and after 1 hr into evacuated 8 ml gas vials. Gases were quantified on a Shimadzu 17A gas chromatograph with electron capture detector (ECD), flame ionization detector (FID), and methanizer (Shimadzu Scientific Instruments, Columbia, MD, USA). As in Delaware, the experiment proceeded for 3 months, after which soils from 0–5 to 10–15 cm depths were collected, a portion stored at −20°C for DNA sequencing, and another aliquot analyzed for porewater biogeochemistry. Measured variables at Alligator were porewater NO_3^-, SO_4^{2-}, total organic carbon (TOC), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), total nitrogen (TN), NH_4^+, PO_4^{3-}, and soil pH, %C, %N and C:N. Samples from Alligator are all manipulated laboratory samples (n = 15).

2.2. Microbial Sequencing and Analysis

Soils were frozen at −20°C until DNA extraction. DNA was extracted from 0.3 g of soil with a Qiagen DNeasy PowerSoil kit following the manufacturer's instructions. PCR was then used to amplify the V4 region of the 16S

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

rRNA gene, following the standard methods of the U.S. Department of Energy Joint Genome Institute (Tremblay et al., 2015). DNA was sequenced on a MiSeq 2000 (Illumina Inc., CA, USA) with paired-end 150 base pair chemistry. Raw data were processed with the iTagger pipeline to quality-filter reads, dereplicate sequences, and cluster sequences into operational taxonomic units (OTUs) at 97% sequence similarity (Tremblay et al., 2015). Taxonomy was assigned using the "assignTaxonomy" function in the *dada2* R package (Callahan et al., 2016), with the SILVA 138.1 taxonomic database (Quast et al., 2013). The *mctoolsr* R package (Leff, 2022) was used to remove chloroplast and mitochondrial DNA and any taxa that were not assigned at least to Bacteria or Archaea at the domain level. We used known taxonomy-function relationships to assign functional guilds of interest for anaerobic biogeochemistry (Hartman et al., 2024), using the "Get_16S_guilds_alt" function in a publicly available R script (https://github.com/cliffbueno/SF_microbe_methane/blob/main/modules/AssignGuilds.R). Sequencing depth was 131,845 ± 3373 SE sequences per sample, and data were rarefied to 31,264 sequences per sample. Only one sample from San Francisco with very few reads (1,296) was dropped. The final sample size analyzed was 133 (Table 1). Sequences were deposited to NCBI GenBank with BioProject ID PRJNA1004999.

2.3. Statistical Analysis

The number of OTUs observed per sample and Shannon diversity were used as microbial alpha-diversity metrics. The effects of site, salinity class (freshwater or oligohaline), and depth (surface 0–5 cm range vs. deeper 5–15 cm range) on alpha-diversity were tested with ANOVA followed by Tukey's post hoc. Microbial community composition was assessed by calculating a Bray-Curtis dissimilarity matrix on square-root transformed abundances, and performing a PERMANOVA test implemented with the "adonis2" function in the *vegan* R package (Oksanen et al., 2022). Within-group multivariate homogeneity of dispersion was tested with PERMDISP implemented with the "betadisper" function in *vegan*. To compare a presence/absence-based metric with the Bray-Curtis metric, we also calculated the Jaccard dissimilarity metric with *vegan*. Unique and overlapping taxa in freshwater and oligohaline salinity classes were calculated with *mctoolsr*. Differences in relative abundances of taxa among the salinity classes were tested with Wilcoxon tests. Indicator species analysis to identify taxa associated with freshwater or oligohaline salinities was performed with the "multipatt" function in the *indicspecies* R package (De Cáceres & Legendre, 2009), with the "r.g" species-site group association function. A phylogenetic tree was built by aligning sequences with MUSCLE (Edgar, 2010) and then building a tree with fasttree (Price et al., 2009), both of which were implemented in QIIME (Caporaso et al., 2010). Nearest taxonomic index (NTI) was calculated for each sample using the "NTI.p" function in the *iCAMP* R package (Ning et al., 2020). NTI is a standardized measure of the phylogenetic distance to the nearest taxon for each taxon in a sample. NTI values >2 or <−2 suggest deterministic processes govern community assembly while NTI values between −2 and 2 suggest dominance of stochastic processes (Stegen et al., 2012). Models of environmental predictors of community composition were tested with distance-based redundancy analysis (dbRDA), implemented with the "capscale" function in *vegan*; the best combination of predictors was selected using backward model selection with the "ordistep" function in *vegan*. Communities were visualized with principal coordinates analysis (PCoA), with environmental vectors fit with the "envfit" function in *vegan*. Spearman correlations between CH_4 flux and chemical variables and certain microbial guild or taxa abundances were calculated and *p*-values corrected with false discovery rate (FDR). All figures were made with either the *ggplot2* (Wickham, 2016) or *pheatmap* (Kolde, 2019) R packages. All analyses were performed with R version 4.2.3 (R Core Team, 2023).

3. Results

All wetland soils measured in this study emitted a net flux of CH_4 to the atmosphere, the amount of which varied by at least three orders of magnitude (Figure 1). Among the five data sets, there were discrepancies in the relationship between salinity and CH_4 fluxes, with 2 positive relationships, 1 negative relationship, and 2 neutral relationships (Figure 1).

Microbial alpha diversity metrics, including OTU richness and Shannon diversity, differed significantly among sites, salinity classes, and depths (Figure 2, Table 2). In two sites, San Francisco and Waccamaw, richness in oligohaline samples was significantly lower than richness in freshwater samples, as was Shannon diversity in San Francisco and Alligator (Tukey HSD $p < 0.05$, Figure 2). Alpha diversity was not significantly affected by salinity class in the Delaware field or lab experiments.

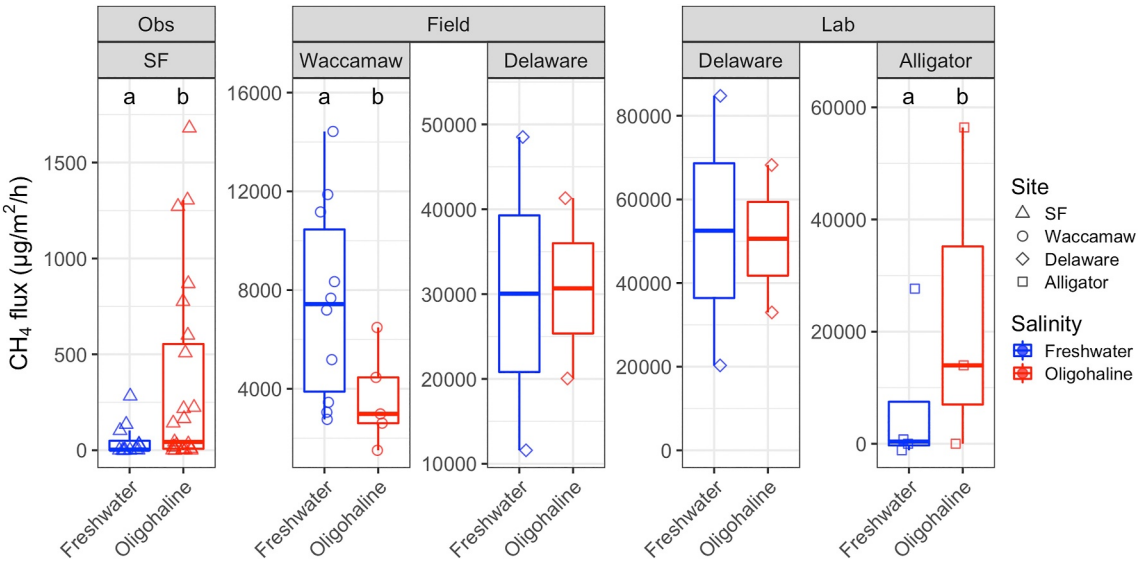

Figure 1. Methane (CH_4) flux from the observational study (Obs), both field experiments, and both lab experiments. Note the difference in *y*-axis scales among panels and that each study used a different method for measuring CH_4 flux, so absolute values of fluxes are not comparable across panels, only within panels. Sample sizes per salinity class per study are 36 for San Francisco, 15 for Waccamaw, 4 for Delaware field, 4 for Delaware lab, and 7 for Alligator (Table 1). Different letters represent significant differences (*t*-test, $p < 0.05$). Data are only shown for cores with microbial data. Data from the full experiments at Waccamaw and Alligator are consistent with results shown here. Data from the full Delaware lab experiment show increased CH_4 flux in the oligohaline treatment.

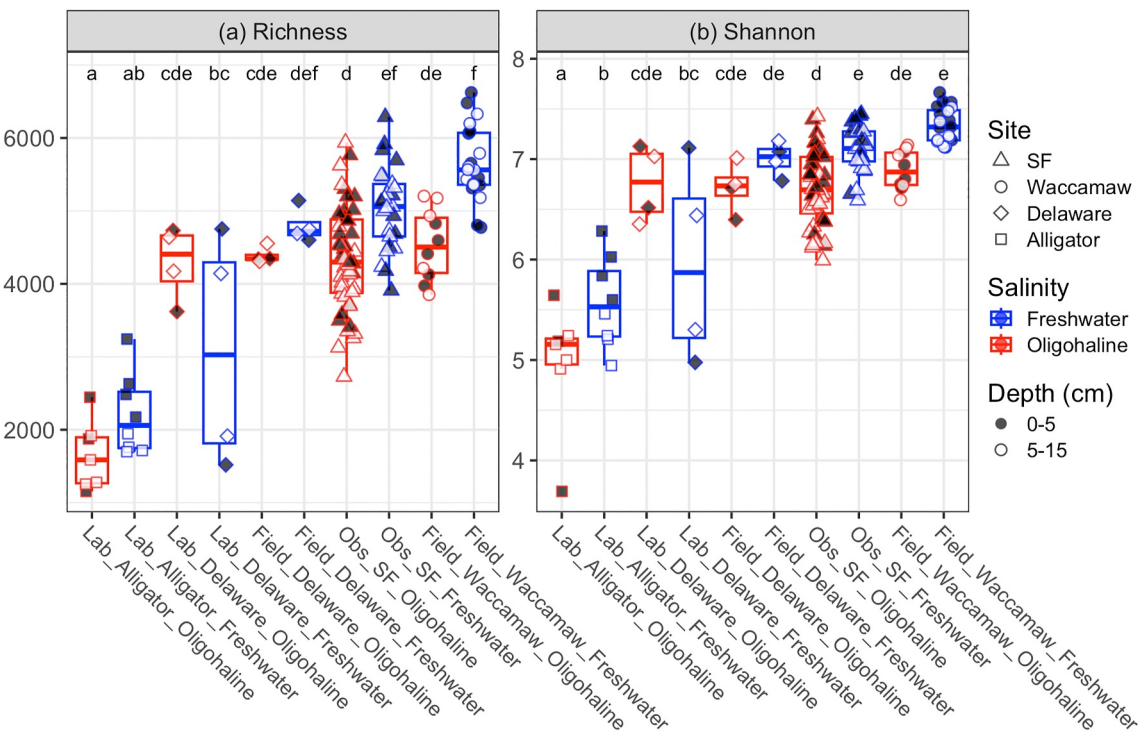

Figure 2. OTU richness (a) and Shannon diversity (b) of bacterial and archeal soil communities across four different sites, two salinity classes, and two depth ranges. DE = Delaware, SF = San Francisco. Sample sizes per salinity class per study are 36 for SF, 15 for Waccamaw, 4 for Delaware field, 4 for Delaware lab, and 8 or 7 for Alligator (Table 1). Different letters represent significant pairwise comparisons within each panel (Tukey posthoc, $p < 0.05$). The *x*-axis is sorted by increasing ASV richness by study (combined freshwater and oligohaline data for each study), with freshwater on the left and oligohaline on the right, for each study. Study type (lab, field, or observational (Obs)) is stated in the *x*-axis labels.

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2
Statistical Results for Microbial Alpha- and Beta-Diversity Across the Whole Data Set

Dep. variable	Ind. variable	F	R^2	P	Test
OTU Richness	Site	80.6	0.66	<0.001	ANOVA (Type II)
	Salinity class	25.1	0.17	<0.001	ANOVA (Type II)
	Depth	4.2	0.03	0.04	ANOVA (Type II)
Shannon	Site	86.3	0.67	<0.001	ANOVA (Type II)
	Salinity class	23.7	0.16	<0.001	ANOVA (Type II)
	Depth	6.9	0.05	0.01	ANOVA (Type II)
B-C Dissimilarity	Site	28.9	0.38	0.001	PERMANOVA
	Salinity class	11.6	0.05	0.001	PERMANOVA
	Depth	3.1	0.01	0.001	PERMANOVA
	CH_4	6.9		0.005	RDA
	Salinity	5.5		0.005	RDA

Note. B-C = Bray-Curtis.

Microbial community composition was primarily driven by site and secondarily by salinity, pH, depth, and suites of other environmental factors that were unique to each site (Figure 3, Table 2). When analyzing all of the data together, samples from each site, including all depths, salinities, and environmental gradients within each site, clustered together, demonstrating a primary influence of site. Communities associated with higher salinities were also associated with lower CH_4 emissions (Figure 3a, "envfit" $p < 0.05$).

To further assess the role of environmental factors within sites, tests were also performed for each site/experiment separately. In San Francisco, a field study with the highest sample size, many environmental variables, including CO_2 flux, CH_4 flux, salinity, bulk density, soil C, N, C:N, NO_3^-, NH_4^+, pH, Cu, Zn, Mn, Fe, PO_4^{3-}, and porewater DOC, and Zn, were correlated with community composition (Figure 3b). Stepwise redundancy analysis model selection suggested that bulk density, soil Zn, pH, Cl^-, C:N, and Mn, and porewater sulfate were the most important variables driving microbial community composition. In the Waccamaw field experiment, results were similar; samples in plots that received seawater additions and thus became oligohaline, and with higher C:N ratios and pH, were significantly different from control freshwater samples, which had higher CO_2 flux, soil N concentrations, net N_2 emissions, and soil oxygen demand (SOD). There was also a

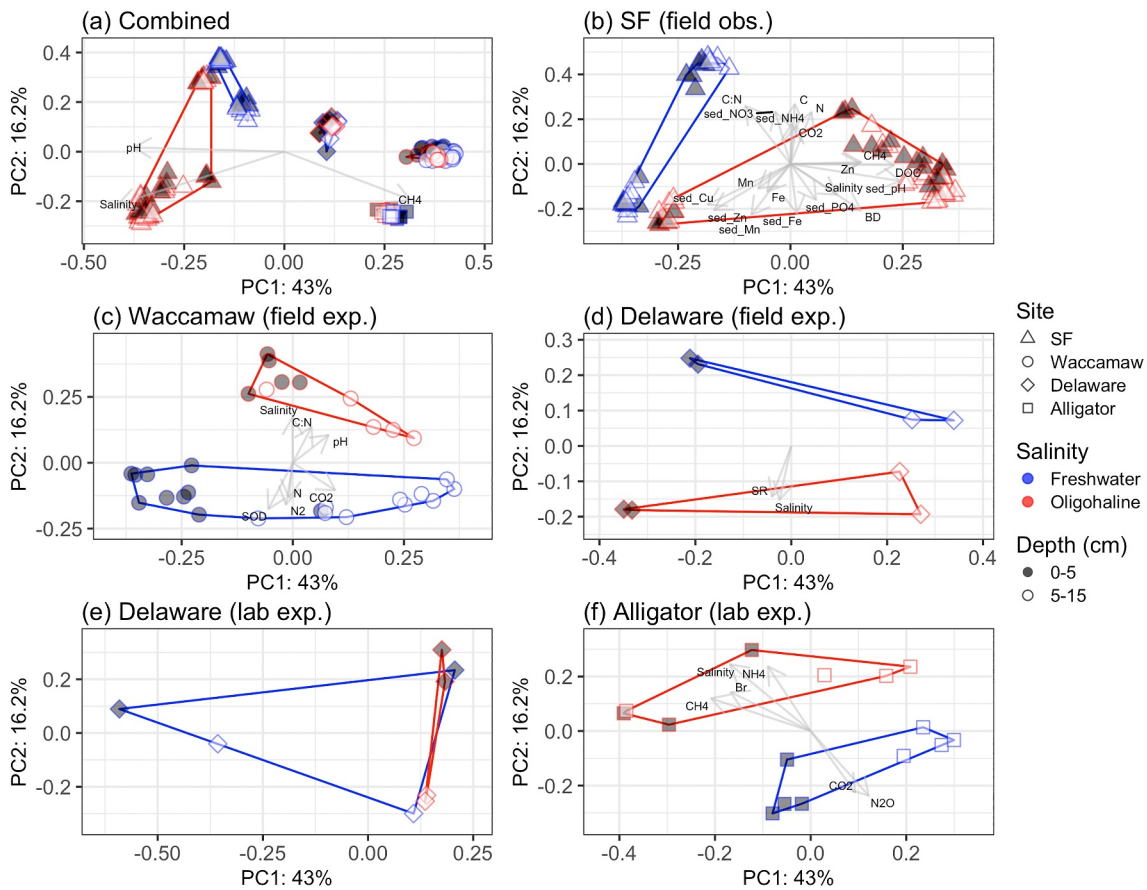

Figure 3. Principal coordinates analysis of Bray-Curtis dissimilarities at the OTU level for bacterial and archeal soil communities for all data from all four estuaries (a), San Francisco Bay field salinity gradient (b), Delaware River estuary field experiment (c), Waccamaw River estuary field experiment (d), Delaware River laboratory incubation experiment (e), and Alligator River estuary laboratory incubation experiment (f). Sample sizes per salinity class per study 36 for SF, 15 for Waccamaw, 4 for DE field, 4 for DE lab, and 8 or 7 for Alligator (Table 1). Note that salinity and CH_4 were the only two continuous variables measured in all five studies (shown as vectors in panel a).

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

significant effect of depth (Figure 3c). The best model predicting community composition contained SOD and CO_2 flux as variables. In the Delaware field transplant experiment, oligohaline samples, associated with increased salinity and sulfate reduction, were significantly different from freshwater samples. The best model predicting community composition only contained salinity as a predictor variable. Depth was also a particularly important factor at this site; samples from the same depth but different salinities were more similar than vice versa (Figure 3d). Laboratory incubations with Delaware wetland soils did not cleanly cluster by depth and salinity class as the other experiments did, due to a high degree of variability in the control (freshwater salinity) samples and a lower sample size (Figure 3e). Community dispersion was not homogenous among the two salinity classes (PERMDISP, $p < 0.05$), indicating this higher degree of variability within the control group. On the other hand, the laboratory incubations with Alligator soils did cluster by salinity class and depth. Samples that received ASW addition making them oligohaline were associated with higher NH_4^+, Br^-, and CH_4 while controls were associated with higher CO_2 and N_2O fluxes (Figure 3f). SO_4^{2-}, PO_4^{3-}, and CH_4 flux were the three variables selected to best predict microbial community composition in stepwise redundancy analysis.

Across the whole data set, the most abundant bacterial phyla were Proteobacteria (mean = 21%), Acidobacteriota (10%), Chloroflexi (8%), Bacteroidota (8%), Desulfobacterota (7%), Firmicutes (7%), Actinobacteriota (6%), Verrucomicrobiota (5%), Planctomycetota (5%), Nitrospirota (3%), Myxococcota (3%), and Crenarchaeota (2%) (Figure 4). Within sites, many of these phyla differed significantly between freshwater and oligohaline samples (Figure 4), with some consistent responses detected in Verrucomicrobiota (negative response in 3/4 sites), Proteobacteria and Myxococcota (negative response in 2/4 sites), and Firmicutes and Chloroflexi (positive response in 2/4 sites) (Figure 4). There were also some key differences in dominant phyla among the sites, with Alligator characterized by a much higher percentage of Acidobacteriota and Firmicutes than the other sites.

The most abundant microbial functional guilds across all samples were sulfate reducing bacteria (mean = 6%), syntrophic sulfate reducing bacteria (2%), ammonia oxidizing bacteria (2%), ammonia oxidizing archaea (2%), nitrite oxidizing bacteria (1%), and type I methane oxidizing bacteria (1%) (Figure 4). All other guilds made up less than 1% of the community on average across all sites, although in some individual samples they had greater relative abundances. Across all samples, methanogens averaged 0.8% relative abundance and methanotrophs averaged 2.7% relative abundance. Most guilds varied significantly among sites and within sites some were differentially abundant between freshwater and oligohaline samples. However, unlike some of the dominant phyla, there were no consistent responses to salinity among guilds across multiple sites (Figure 4).

There were 220 methanogen OTUs: 146 hydrogenotrophic OTUs, 29 acetoclastic OTUs, 35 methyl-reducing OTUs, and 10 mixotrophic OTUs. 44 methanogen OTUs were found in at least two sites. Methanogen community composition varied among the 4 sites (Figure S3 in Supporting Information S1). Hydrogenotrophs were the most abundant methanogen guild across the whole data set, followed by methyl reducers, acetoclasts, and mixotrophs, mirroring the trend in methanogen guild OTU richness (Figure 4, Figure S3 in Supporting Information S1). Hydrogenotrophs, acetoclasts, and methyl-reducers were generally positively associated with CH_4 flux in all sites (Figure 5). These three guilds also tended to be positively correlated with salinity, while mixotrophic methanogens had mixed relationships among the sites (Figure S3 in Supporting Information S1). Of the 13 methanogenic families identified across the whole data set, Methanobacteriaceae (containing hydrogenotrophs and methyl-reducers), was the most abundant, followed by Methanoregulaceae (hydrogenotrophs), Methanosaetaceae (acetoclasts), Methanomassiliicoccaceae (methyl-reducers), and Methanosarcinaceae (mixotrophs containing hydrogenotrophs, acetoclasts, methylotrophs, and methyl-reducers). Methanofastidiosaceae (hydrogenotrophs and methylotrophs (Nobu et al., 2016)) increased in abundance with salinity in both field studies (Figure S2 in Supporting Information S1). The ratio of methanogens to methanotrophs (MG:MT) was positively correlated with CH_4 flux in three sites, but only significantly in the higher sample size site (San Francisco) (Figure 5).

There were 445 methanotroph OTUs: 394 MOB_I OTUs, 24 MOB_II OTUs, 21 MOB_IIa OTUs, and 6 ANME OTUs. Methanotroph community composition varied among the 4 sites (Figure S4 in Supporting Information S1). The aerobic type I methane oxidizing bacteria (MOB_I) were the most abundant methanotrophic guild across the whole data set, followed by MOB_II, MOB_IIa, and ANME. The most abundant methanotrophic genera included *Methylocystis*, *Crenothrix*, *Methyloceanibacter*, and *Methylocaldum*. Some taxa consistently responded to salinity in the two field studies; in both San Francisco and Waccamaw, *Methyloceanibacter* increased with salinity while *Methylomagnum* and *Candidatus Methylospira* decreased with salinity (Figure S4 in Supporting

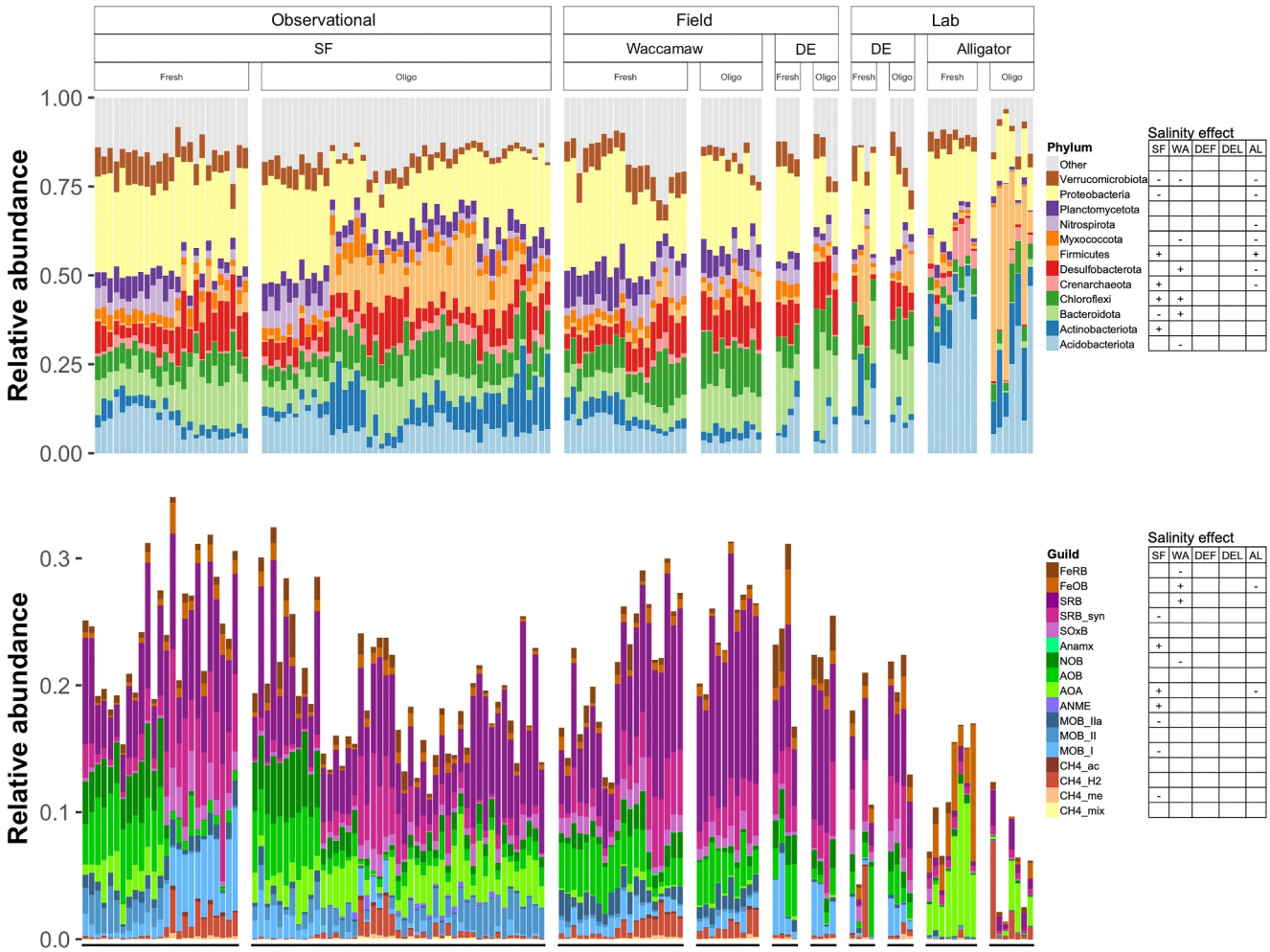

Figure 4. Relative abundance of the top 12 phyla (top panel) and functional guilds (bottom panel), and whether there is a significant positive (+) or negative (−) effect of salinity on the phylum or guild. Relative abundances in the top panel sum to 1, as all other taxa are included in the "Other" category. Relative abundances in the bottom panel are aggregated counts of OTUs that are assigned to those guilds. DEF = Delaware field experiment; DEL = Delaware lab experiment. Each column represents an individual sample, but sample IDs are omitted from the *x*-axis label for clarity. Functional guilds are: FeRB = iron-reducing bacteria, FeOB = iron-oxidizing bacteria, SRB = sulfate-reducing bacteria, SRB_syn = syntrophic sulfate-reducing bacteria, Anamx = anaerobic ammonia oxidizing bacteria, NOB = nitrite-oxidizing bacteria, AOB = ammonia-oxidizing bacteria, AOA = ammonia-oxidizing archaea, ANME = anaerobic methane-oxidizing archaea, MOB_IIa = type IIa methane-oxidizing bacteria, MOB_II = type II methane-oxidizing bacteria, MOB_I = type I methane-oxidizing bacteria, CH_4_ac = acetoclastic methanogens, CH_4_H2 = hydrogenotrophic methanogens, CH_4_me = methyl-based methanogens, CH_4_mix = mixotrophic methanogens.

Information S1). The four methanotrophic guilds had variable relationships with CH_4 flux among the four sites (Figure 4). AO:NOB ratios were not significantly correlated with methanotroph abundances or and methanogen: methanotroph ratios except in San Francisco, where they were positively correlated, and Waccamaw, where the ratios were negatively correlated (Figure S5 in Supporting Information S1).

At the OTU level, there was a diverse group of 48 OTUs that could be considered strong indicators of either freshwater (n = 7) or oligohaline (n = 41) salinity conditions, with FDR corrected p-values < 0.05 and indicator correlation coefficients >0.45 (Figure S6 in Supporting Information S1). Freshwater indicator taxa included OTUs from 7 different phyla but none were classified to genus. Oligohaline indicator OTUs included taxa from 13 different phyla and encompass some known genera and functional guild classifications such as the sulfate reducers *Desulfatiglans* and *SEEP-SRB1* and the nitrifiers *Nitrospira*, as well as taxa only identified to broader taxonomic levels.

Several biogeochemical variables and microbial guilds were significantly correlated with methane flux (Figure 5). In terms of salinity and alternative electron acceptors expected to be negatively correlated with CH_4 flux, NO_3^-

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

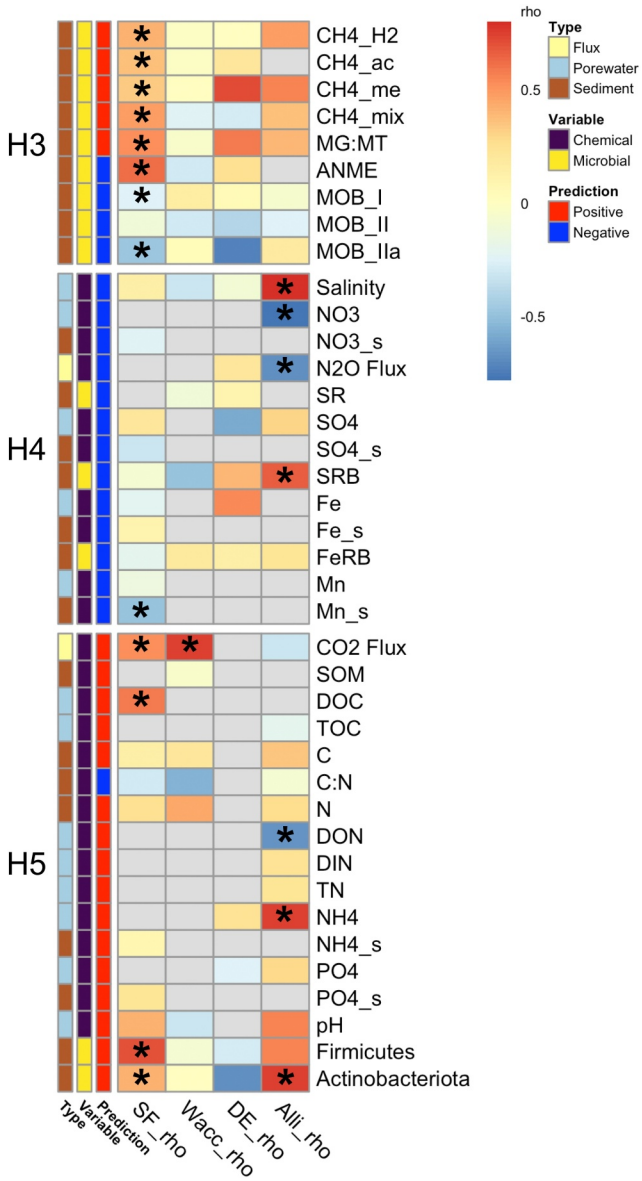

Figure 5. Correlations between chemical and microbial variables and methane flux. Rows are annotated by type (flux, porewater or soil), variable (chemical or microbial), and the predicted relationship with methane (positive or negative). Blocks are labeled with hypotheses H3, H4, or H5, which correspond to the stated hypotheses about methanogens/methanotrophs, alternate electron acceptors, and overall decomposition. The heatmap shows Spearman's rho values and asterisks indicate if the test was statistically significant ($p < 0.05$). Guild abbreviations are given in Figure 4 caption. Gray cells indicate data not present. Data shown here from DE are from the field experiment only. MG:MT = methanogen: methanotroph ratio, SR = sulfate reduction rate; for chemical abbreviations see the methods text.

and N_2O flux were negatively correlated with CH_4 flux in the Alligator incubation, and Mn was negatively correlated with CH_4 flux in San Francisco. Salinity as a continuous variable (as opposed to categorical in Figure 1) was significantly *positively* correlated with CH_4 flux in Alligator incubations and otherwise uncorrelated. For metrics of soil fertility and decomposition rates, CO_2 flux, total soil C and N, and porewater NH_4^+ concentrations were generally positively correlated with CH_4 flux, though not in all sites in which the variable was measured. pH was not significantly correlated with CH_4 flux in any of the three sites in which it was measured. Several variables were significantly correlated with salinity, including a positive correlation between NH_4^+ and salinity in both Delaware and Alligator, but contrasting relationships between salinity and N_2O flux at those two sites (Figure S2 in Supporting Information S1).

A key contrasting result was an increase in CH_4 flux following ASW addition in the Alligator River estuary forested wetland laboratory incubations, and lack of increased CH_4 flux in week 12 of the Delaware River estuary laboratory incubations (Figure 1), both of which were performed using similar methodology. Soils from Alligator have low pHs (<5.5, Figure S7 in Supporting Information S1) and were dominated to a much greater extent by Acidobacteriota (Figure 4). In terms of the dominant methanogenic taxa, different OTUs within the Methanobacteriaceae family were dominant at each site, and these different OTUs responded differently to the +ASW treatment. Two OTUs at Alligator increased with ASW addition and were associated with greater CH_4 fluxes (Figure 6).

The percent of shared OTUs among salinity classes within each site was 44%–45% in laboratory experiments and 34%–40% in field samples (Figure S8 in Supporting Information S1). Nearest taxon index (NTI) was >2 in 131 of 133 samples (Figure S9 in Supporting Information S1). NTI was significantly lower in oligohaline samples than freshwater samples in San Francisco, Waccamaw, and Alligator but not Delaware (Figure S9 in Supporting Information S1). Dissimilarity between freshwater and oligohaline samples versus samples in the same salinity class was greater for both Bray-Curtis and Jaccard metrics; Cohen's *d* effect size was slightly greater for Jaccard dissimilarity, but this was not affected by field or lab conditions (Figure S10 in Supporting Information S1).

4. Discussion

4.1. Microbial Alpha-Diversity (H1)

Salinity is expected to decrease the alpha-2024 diversity of freshwater microbial communities as increased salinity creates osmotic stress that requires organisms to either produce compatible solutes or, at extreme salinities, have specialized systems to function at high internal salt concentrations (Gunde-Cimerman et al., 2018; Oren, 2013). While there are many organisms that can tolerate a range of salinities, oligohaline salinities are high enough to cause stress or mortality in those organisms that have not evolved the ability to cope with a range of salinities (Georges et al., 2019). Our data partially support this hypothesis, as we found a negative effect of oligohaline salinity on richness in San Francisco and Waccamaw; however, richness in Delaware and Alligator was not affected. Decreases in bacterial richness have been found elsewhere, such as in freshwater streams affected by mining-induced salinity (Vander Vorste et al., 2019) and along salinity gradients in coastal wetlands (Zhao et al., 2020). In contrast, slight increases in salinity can also cause an increase in richness, which has been found in Louisiana wetlands up to 3.5 ppt salinity (Jackson & Vallaire, 2009), in Tibetan lakes up to 1 ppt salinity (Wang et al., 2011), and in Chinese soils exposed to up to

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

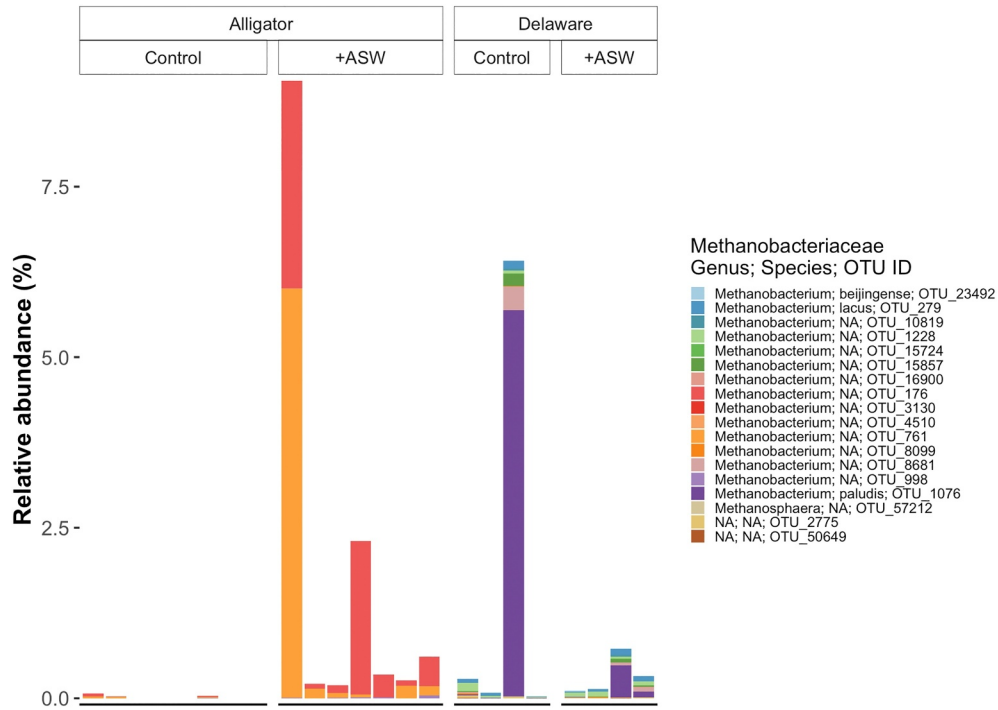

Figure 6. Methanobacteriaceae percent relative abundances for each individual sample in the laboratory incubation experiments conducted with wetland soils from the Alligator River and Delaware River estuaries.

3.33 ppt irrigation water (Chen et al., 2017). Other work has suggested that bacterial communities are resistant to changes up to 3 ppt in salinity (Berga et al., 2017).

4.2. Microbial Beta-Diversity and Taxa (H2)

Microbial community composition is strongly linked to enzyme activity and is thus important to understand with respect to biogeochemistry (Morrissey, Gillespie, et al., 2014). Globally, salinity is a primary determinant of bacterial and archeal community composition (Auguet et al., 2010; Lozupone & Knight, 2007), so it was not surprising to find differences between freshwater and oligohaline communities in all five studies here, although the effect of salinity was secondary to the effect of site (Figure 3). The broad differences in communities among the four sites are likely a product of different site histories, plant communities, and biogeochemistry (Table 1, Figure S7 in Supporting Information S1). Despite these differences, there were many (50 phyla, 121 classes, 237 orders, 264 families, 291 genera) shared taxa among the four sites (Figure S11 in Supporting Information S1). The indicator species analysis also suggested that there are some taxa shared across the sites that consistently associate with either freshwater or oligohaline salinities. A high proportion of Proteobacteria was found in all sites and is consistent with other studies in coastal wetlands (Dang et al., 2019; Jackson & Vallaire, 2009; Zhao et al., 2020). The decline in Verrucomicrobiota in oligohaline sites has also been observed across the Baltic Sea salinity gradient (Herlemann et al., 2011) and demonstrates a possible preference of this phylum for non-saline environments, although some members have been cultured at seawater salinities (Schlesner et al., 2006). Myxococcota have been identified as key components of tidal freshwater wetlands elsewhere (Morina & Franklin, 2022), and have been shown to decline with increased salinity (Zhao et al., 2023), in concordance with our results. While we found positive effects of salinity on the relative abundance of Chloroflexi, another common freshwater and estuarine wetland phylum (Huang et al., 2022; Ikenaga et al., 2010), results in other studies have shown mixed responses to low-level salinity (Zhang et al., 2021; Zhao et al., 2020). Firmicutes, the other major phylum with consistent responses to salinity in more than one site, are also dominant members of other freshwater wetlands and have been shown to respond positively to salinity across estuarine (Zhang et al., 2021), groundwater (Sang et al., 2018), and upland soil (Van Horn et al., 2014; Zheng et al., 2017) salinity gradients.

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

4.3. Methanogens and Methanotrophs (H3)

While tidal wetlands have high functional redundancy for CO_2 production, there are stronger links between CH_4 production and microbes due to the conserved nature of methanogenic archaea (Morrissey, Berrier, et al., 2014). All four methanogenic guilds distinguishable with taxonomic data were present in the data set, and the most abundant methanogens were hydrogenotrophs and acetoclasts, suggesting that these pathways of methanogenesis dominate in these wetlands. All guilds were generally positively associated with CH_4 flux, except for mixotrophs in Waccamaw and Delaware. This discrepancy is likely explained by other controls on CH_4 flux at play in the samples with high mixotroph abundance. In the Delaware River Estuary, isotope-based measurements demonstrated that acetoclastic methanogenesis dominated over hydrogenotrophic methanogenesis, although both contributed to the observed CH_4 flux; methyl-based methanogenesis was not estimated (Weston et al., 2011, 2014). Acetoclastic methanogens identified in the Delaware 16S data include the pure acetoclastic genus *Methanosaeta* and the mixotrophic genus *Methanosarcina*. On the other hand, pure acetoclasts were absent from Alligator; acetoclastic methanogenesis may still contribute to CH_4 flux there via mixotrophic Methanosarcinaceae, which were present and positively correlated with CH_4 flux. Acetoclasts have been suggested to increase with low salinity (Berrier et al., 2022); in our data sets this was only the case in San Francisco. Though not as abundant, taxa in the methyl-reducing Massilliicococcales order were present in all sites and positively correlated with CH_4 flux (though only significantly in San Francisco). The relative contribution of methyl-based methanogenesis is expected to increase with increasing salinity and in marine to hypersaline conditions due to the non-competitive substrates (e.g., betaine, trimethylamine) produced as osmolytes (Bueno de Mesquita et al., 2023; Oren, 1999; Zhou et al., 2021), but is perhaps less relevant at low salinities. In publicly available metagenomic data sets from freshwater and coastal wetlands, the abundance of archeal genes involved in different methanogenesis pathways followed similar trends of a gene involved in acetoclastic methanogenesis (*cdhD*) being the most abundant, followed by hydrogenotrophic (*frhA*) and methylotrophic genes, with higher abundances in freshwater wetlands (Bueno de Mesquita et al., 2023). The ratio of methanogens to methanotrophs (MG:MT) was positively correlated with CH_4 flux in most sites, as seen elsewhere (Rey-Sanchez et al., 2019; Zhang et al., 2019), suggesting that this is an important microbial metric to calculate. Furthermore, MG:MT was positively correlated with salinity in Alligator soils, in line with the increase in CH_4 flux there.

A substantial proportion of the total CH_4 produced in methanogenic environments is consumed by methanotrophs (i.e., methane oxidizers) before it is emitted to the atmosphere, making them an important factor in regulating net CH_4 fluxes. In marine soils the amount has been estimated to range from 43% to 85% (Reeburgh, 2007). Four methanotrophic guilds were present at each site, yet their abundances were not consistently negatively correlated with CH_4 flux, highlighting the complexity of factors that contribute to observed CH_4 fluxes. MOB_I and MOB_IIa were negatively correlated with CH_4 flux in San Francisco, however, and their higher abundances may have suppressed emissions in a wetland complex with higher methanogen abundances (Hartman et al., 2024). Prior work has shown that both aerobic and anaerobic methanotrophy declined with salinity, with particular sensitivity of aerobic methanotrophy (Dalal et al., 2008). In our data set, aerobic bacterial methanotrophs (MOB_I, MOB_II, MOB_IIa) were much more abundant than anaerobic archeal methanotrophs (ANME). Two of the three aerobic guilds did decline with salinity, but only in San Francisco (Figure 3), so our results only weakly agree with prior work (Dalal et al., 2008). Methanotroph communities differed among the sites (Figure S4 in Supporting Information S1), with key abundant taxa including *Crenothrix* and *Methylocystis*. *Crenothrix* spp. are also dominant methanotrophs in stratified lakes (Oswald et al., 2017), while *Methylocystis* spp. are also dominant in peatlands (Chen et al., 2008). While some *Methylocystis* species are inhibited by salinities >5 ppt (Dedysh et al., 2007), *Methylocystis* was still abundant in Waccamaw oligohaline samples. *Methylospira* and *Methylomagnum* declined with salinity in both field studies, while *Methyloceanibacter*, containing known marine methanotrophs (Takeuchi et al., 2014), not surprisingly increased with salinity, highlighting the taxa-specific environmental preferences of methanotrophs to different salinities. This may explain the lack of relationship between aggregated guild abundances and salinity, as at the guild level, all of the potentially positively and negatively responding taxa are summed.

Previous work in rice paddies has suggested links between nitrogen cyclers and methanotrophs (Bodelier, 2011; Bodelier & Laanbroek, 2004; Bodelier & Steenbergh, 2014). AOA and AOB perform the first step of nitrification, oxidizing NH_3 to NO_2^-. Then NOB perform the second step, oxidizing NO_2^- to NO_3^-. Thus, the combined activities of AO and NOB create NO_3^-, which could suppress CH_4 flux due to the stimulation of nitrate reducers which can outcompete methanogens or by the inhibition of methane oxidizers. Alternatively, AO and NOB could

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

increase CH_4 flux due to increased inorganic N available for organic carbon-producing plant growth and microbial growth. Furthermore, a high ratio of AO to NOB could indicate nitrite buildup, which, along with other forms of inorganic N, could be toxic to methanotrophs in excess (Bodelier & Laanbroek, 2004; Dunfield & Knowles, 1995). Our abundance data for AO:NOB and methanotrophs did not support this hypothesis of inhibition, with variation among sites and a lack of relationship in most cases. While influenced by agricultural runoff, our sites are unfertilized and therefore have lower inorganic nitrogen levels than the agricultural settings studied in previous work on inorganic N inhibition of methanotrophs (Cai et al., 2007).

While the results on methanogens and methanotrophs presented here are limited to 16S rRNA marker gene sequencing, they agree with shotgun metagenomic data from two of the sites—San Francisco and Alligator (Bueno de Mesquita et al., 2024; Hartman et al., 2024). In particular, relative abundances of methanogens and methanotrophs from 16S rRNA gene PCR amplicons matched relative abundances of 16S rRNA genes extracted from metagenomes with mTAGs (Bueno de Mesquita et al., 2024; Salazar et al., 2021), suggesting limited effects of any potential primer biases. Furthermore, relative abundances of *mcrA* and *amoA*, key marker genes of methanogenesis and methanotrophy, respectively, annotated from metagenomic data by sequence homology (Morgan-Lang et al., 2020), were strongly and significantly correlated with the relative abundances of methanogens and methanotrophs extracted taxonomically from 16S PCR amplicon data. While we did not perform qPCR of these key genes, the homology-based approach with shotgun metagenomic data was actually more strongly correlated with 16S taxonomic relative abundances than *in silico* PCR, which was assessed for *mcrA* and *amoA* (Hartman et al., 2024).

4.4. CH_4, Salinity, Alternative e−Acceptors (H4)

While methane fluxes are generally expected to decrease with low salinity, as shown by several meta-analyses (Al-Haj & Fulweiler, 2020; Bartlett et al., 1987; Poffenbarger et al., 2011) and studies (Berrier et al., 2022; Chambers et al., 2011, 2013; Marton et al., 2012; Neubauer, 2013), data from a diverse range of individual sites and experiments show that salinity/methane relationships are highly variable and include positive, negative, and neutral relationships (Ardón et al., 2018; Helton et al., 2019). The degree of salinity and length of exposure to salinity are important, with different relationships seen above and below 7.5 ppt (Wang et al., 2017) and over different time periods; for example, previous work at Waccamaw demonstrated that long-term saltwater intrusion reduced both CO_2 and CH_4 flux, while short-term exposure increased CO_2 but decreased CH_4 (Neubauer et al., 2013). This is also an important source of variation among our 5 studies, where samples had been exposed to salinity for decades (San Francisco), 3 years (Waccamaw), 1 year (Delaware field), and 3 months (Delaware lab, Alligator lab) (Figure 5). Elsewhere, after 1 week of saltwater exposure CO_2 increased but CH_4 decreased (Dang et al., 2019). These results may be attributable to microbial community composition changes and adaptations. Intrusion events followed by recovery from salinity involve dynamic microbial interactions that affect carbon cycling (Berrier et al., 2022). Temporary changes in salinity are expected to select for different microbial taxa (e.g., adapted to a range of salinities) compared to long-term changes in salinity, which are expected to select for taxa optimized for growth at the given salinity (Chambers et al., 2013). For example, when freshwater and marine microbial communities were mixed, the community shifted toward the marine community composition, and when freshwater communities were exposed to sterile marine water, more freshwater taxa were lost from the community than saltwater taxa were lost when saltwater communities were exposed to freshwater (Rocca et al., 2020). Another important variable is hydrologic setting—in permanently flooded soils, low salinity suppressed methanogenesis, while in intermittently flooded soils, low salinity increased methanogenesis (Helton et al., 2019). Flooded samples have greater abundances of both methanogens and methanotrophs than non-flooded samples (Rey-Sanchez et al., 2019). In our data set all samples were continuously covered with water except for Delaware laboratory incubations which underwent a simulated tidal cycle of 6 hr of flooding and 6 hr of air exposure. We sought to use additional data on microbial community composition and biogeochemistry to explain discrepancies in salinity/methane relationships.

Seawater exposure is expected to increase the availability of terminal electron acceptors for microbial growth, which affects the pathways by which organic matter is degraded (Sutton-Grier et al., 2011; Weston et al., 2006). In particular, nitrate, sulfate, iron, and manganese are key electron acceptors for microbial metabolism in anaerobic environments, and in tidal freshwater marshes specifically (Lovley, 1991; Megonigal et al., 2019; Weston et al., 2006). We hypothesized that salinization by seawater would suppress CH_4 flux due to competition between organisms reducing those electron acceptors and methanogens. Furthermore, these acceptors are used by methanotrophs during methane oxidation, whose activity would further decrease CH_4 flux (Guerrero-Cruz

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

et al., 2021). Nitrate was negatively correlated with CH_4 emissions at Alligator, consistent with what has been shown in rice paddies (Cai et al., 2007). This is also consistent with findings that increases in NO_3^- increase denitrification rates (Morrissey & Franklin, 2015), and denitrifier abundances increase with low salinity (Franklin et al., 2017). Fluxes of N_2O, a product of denitrification of nitrate, were significantly negatively correlated with CH_4 flux at Alligator, consistent with potential competitive interactions between denitrifiers and methanogens. Of the alternative electron acceptors, sulfate has been the most widely implicated as a control on methanogenesis in freshwater (Lovley & Klug, 1983) and estuarine (Oremland & Polcin, 1982) environments. However, here we find no support for suppression of CH_4 production by sulfate at oligohaline salinities in the sites studied. Sulfate-reducing bacteria (SRB) were the most abundant microbial functional guild and several sulfate reducing taxa were indicators of oligohaline conditions. However, SRB were actually *positively* correlated with CH_4 flux in Alligator soils. Relationships between iron and manganese and salinity are less clear; both increased with salinity in our data sets, but this could also be caused by changes in oxidation states. However, there is no evidence in our data that increased Fe-reducing bacteria led to suppression of methanogenesis. Elsewhere, salinity has initially temporarily increased iron reduction rates, but this effect diminished over time (Weston et al., 2006). Another alternative electron acceptor in wetland soils is humic substances, which are used by certain anaerobic methanotrophs (ANME) (Bai et al., 2019), certain sulfate reducers (Cervantes et al., 2002), and other taxa (Coates et al., 1998). The abundance of humic substances could suppress CH_4 emissions by either increasing activity of ANME (which consume CH_4), increasing the activity of SRB and other humic substance reducers that can outcompete methanogens, or by causing methanogens to switch their metabolism from methanogenesis to humic substance reduction, which does not produce methane (Valenzuela & Cervantes, 2021). We did not quantify humic and phenolic compounds in our studies, but we hypothesize they could be important in the forested and more acidic Alligator site, as $SUVA_{254}$, a common metric of aromaticity and humics in water, was negatively correlated with CH_4 flux earlier in the same experiment on which we report here (Ardón et al., 2018); this remains an important avenue for future research.

While we only present results here from week 12 of the lab experiments, for which there was microbial data available, results from Alligator (increase in CH_4 following artificial seawater addition) were consistent with the entire time course of the experiment (Ardón et al., 2018). CH_4 emissions were significantly elevated starting at day 21 of the experiment and continuing until the experiment's conclusion on day 112. Although the limited amount of flux data available from cores with microbial data (week 12) from Delaware did not show elevated CH_4 flux (Figure 1), the complete time series of that experiment showed increased CH_4 emissions within 1 week after artificial seawater addition and continuing for 5 months (Weston et al., 2011).

4.5. CH_4 and Decomposition (H5)

There are multiple mechanisms by which low salinity can directly and indirectly affect decomposition rates in general, not just methanogenic decomposition. This can impact the directionality of salinity/C mineralization relationships, which have been shown to be highly variable in studies that have quantified either gas flux, enzymatic activities, or mass loss (Luo et al., 2019). For example, low salinity has had positive (Baldwin et al., 2006; Chambers et al., 2011, 2013, 2014; Liu et al., 2017; Marton et al., 2012; Morrissey, Gillespie, et al., 2014; Neubauer, 2013; Saviozzi et al., 2011; Weston et al., 2006, 2011) and negative (DeLaune et al., 1983; Kelley et al., 1990; Krauss & Whitbeck, 2012; Nyman & DeLaune, 1991; Smith et al., 1983; Ury et al., 2022; Wilson et al., 2015) effects on CO_2 production, positive (Cunha et al., 2000; Morrissey, Gillespie, et al., 2014) and negative (Jackson & Vallaire, 2009) effects on enzymatic activities, and a negative effect on mass loss (Roache et al., 2006). Saltwater intrusion affects organic carbon production and solubility, which indirectly affect mineralization by controlling availability (Neubauer et al., 2013). Low salinity can decrease plant productivity (Chamberlain et al., 2020) and C solubility (Ury et al., 2022), which would decrease overall mineralization rates. In addition to influencing carbon solubility, seawater can affect inorganic N (particularly ammonium) and P sorption, liberating nutrients, which in turn affect microbial activity and decomposition rates (Ardón et al., 2013; Weston et al., 2010; Zhou et al., 2017). Low salinity can either increase (Portnoy & Giblin, 1997) or decrease (Ury et al., 2022; Wang et al., 2017) pH, which similarly affects the solubility of compounds, the sorption of phosphorus, and decomposition rates. pH is also a strong driver of microbial community composition in both terrestrial (Lauber et al., 2009) and aquatic (Sadeghi et al., 2021) ecosystems. Lower pH is associated with, and can be driven by, a higher concentration of recalcitrant humics and phenolics that could slow decomposition rates and methanogenesis, as discussed above (Valenzuela & Cervantes, 2021).

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

We predicted that regardless of how they were affected by salinity, CH_4 fluxes would be coupled with CO_2 flux and metrics of organic C, which should fuel metabolic pathways producing CO_2 and CH_4 (Oikawa et al., 2017; Seo et al., 2014; Sutton-Grier et al., 2011). We also predicted positive correlations between CH_4 flux and ammonium and phosphate concentrations, pH, and the relative abundance of Firmicutes and Actinobacteriota. Ammonium and phosphate are important nutrients for microbial growth, low pH may inhibit certain methanogens (Van Kessel & Russell, 1996), and Firmicutes and Actinobacteriota are known to degrade complex carbon sources, which may supply substrates for methanogenesis (Gavande et al., 2021; Hartman et al., 2024). There was mixed support for these predictions. CO_2 and CH_4 were indeed coupled in two field study sites (San Francisco, Waccamaw), but not in Alligator. Notably, although CO_2 is generally produced in much higher quantities than CH_4, it is also produced during acetoclastic and methylotrophic methanogenesis, but not hydrogenotrophic methanogenesis and the most abundant methanogens at Alligator were hydrogenotrophs. Different C, N, and P variables were measured among the sites/experiments, but there were some instances of positive correlations with CH_4 flux, such as DOC in San Francisco, and NH_4^+ in Alligator soils. Ammonium can either increase or decrease CH_4 emissions based on the balance between its effects at the plant/ecosystem level, the microbial community level, and the biochemical level (Schimel, 2000). In Alligator soils, it was associated with increased CH_4 flux, possibly due to increased N available for plant and methanogen growth (Ardón et al., 2018). Phosphate was measured in two sites but was not significantly correlated with CH_4 flux, suggesting that it does not exert a strong influence on CH_4 in these coastal wetlands. Notably, these sites are likely not phosphate limited, which may partially explain this lack of effect; in P-limited systems phosphate abundance may be more important (Herbert et al., 2020; Ket et al., 2011). Methanogenesis relies on upstream depolymerization of polymeric organic matter, as well as degradation of fatty acids such as butyrate (Berrier et al., 2022). In this way methanogenesis is coupled to both photosynthesis and complex carbon degrading microorganisms. Metagenomic data has demonstrated positive correlations between hemicellulose and cellulose degrading genes and CH_4 flux, and that Firmicutes and Actinobacteriota were the dominant taxonomic groups containing those genes (Hartman et al., 2024). Firmicutes and Actinobacteriota were associated with increased CH_4 flux at both San Francisco and Alligator. They have also been implicated in the depolymerization of rice straw, particularly in cellulose and hemicellulose degradation, fueling methanogenesis in rice paddies (Gavande et al., 2021; Kausar et al., 2011); Actinobacteriota were also associated with methane production in *Sphagnum* peat bogs (Pankratov et al., 2006). A lack of relationship in Delaware and Waccamaw could be due to the lower overall abundance of Firmicutes in these sites; other taxa, including those in the Proteobacteria and Bacteroidetes phyla, may be responsible for complex C degradation in those locations.

4.6. Site-Specificity

What could be driving such discrepancies in CH_4/salinity relationships among the sites/experiments? One clear difference is the low pH and high abundance of Acidobacteriota and Firmicutes in Alligator laboratory-incubated soils compared to Delaware laboratory-incubated soils (Figure 3, Figure S7 in Supporting Information S1). This is likely driven by broad differences in plant community composition between the sites, which affects organic carbon quality and quantity which in turn affect microbial communities and decomposition rates (Bueno de Mesquita et al., 2019; Megonigal et al., 2019; Shahbaz et al., 2017; Sutton-Grier et al., 2011). However, another laboratory experiment with soils from a tidal forested wetland similar to Alligator showed a decrease in CH_4 production with seawater addition, highlighting the need for more studies in forested wetlands to be able to make generalizations (Marton et al., 2012). Alligator also notably had the smallest and least rich methanogen population in all samples, particularly in oligohaline samples (Figure S4 in Supporting Information S1). While humic substances were not quantified, they are expected to be higher in the acidic Alligator wetland compared to Delaware Estuary wetlands, and this could suggest another mechanism for increases in CH_4 flux with salinity at that site, if salinity decreases the concentration of humics.

There is also support for the idea that site-specific microbial consortia that are functionally relevant have different responses to low salinity. While many broader taxonomic groups were shared among the four sites, relatively few were shared at the OTU level (Figure S11 in Supporting Information S1). A case in point are the methanogens in the most abundant methanogen family, Methanobacteriaceae (Figure 6). Most Methanobacteriaceae reads were from the hydrogenotrophic *Methanobacterium* genus, but even within the genus there were different OTUs and some evidence for different responses to oligohaline salinity. The idea that different species in the same genus, or even different strains of the same species, have different environmental preferences is not new, especially when

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

considering pathogens (Thelaus et al., 2018), and has been greatly expanded upon in recent years with the help of full genome sequencing and metabolic modeling. For example, metabolic modeling of 55 *Escherichia coli* strains showed differences in niches among the strains (Monk et al., 2013). Such site-specific OTU niche differences and salinity responses, particularly of taxa involved in CH_4 cycling, can then contribute to discrepancies in CH_4/salinity relationships.

4.7. Field Versus Lab Settings

Another potential source of discrepancy in CH_4/salinity relationships among the five data sets examined here could arise from differences in field and laboratory experimental conditions. Field samples are, not surprisingly, characterized by greater richness and diversity than the laboratory samples taken after 3 months in the laboratory (Figure 2). Laboratory experiments also did not include plants and therefore do not take into account changes in C inputs as plants grow, release exudates, and senesce. To test for differences in the ecological dynamics between laboratory and field samples, we analyzed taxa overlap and nearest taxon index (NTI), and compared a presence/absence metric (Jaccard) with an abundance-based metric (Bray-Curtis). Field samples are expected to have more unique taxa in freshwater and oligohaline conditions than samples incubated in the lab; field samples are mixed with the surrounding environment and microbes can disperse into the sampled soil from the surrounding environment, whereas samples incubated in the laboratory are cut off from the regional species pool after they are initially collected from the field. Dispersal and immigration are key processes in microbial community assembly (Sloan et al., 2006). Residence times of estuarine microbes in the field are on the order of days to weeks, indicating the potential for dynamic temporal turnover (Crump et al., 2004).

As expected, laboratory experiments had a higher degree of overlap in OTUs among salinity classes (Figure S8 in Supporting Information S1). However, differences in community composition among salinity classes were similar when using presence/absence and abundance-based metrics, and there were no differences among field and lab samples (Figure S10 in Supporting Information S1). Furthermore, virtually all samples, whether from field or lab, were dominated by deterministic assembly processes (Figure S9 in Supporting Information S1), even in the field where stochasticity could have potentially played a larger role due to dispersal and immigration. The NTI values >2 suggest phylogenetic clustering in most samples. Overall this suggests that similar ecological dynamics are at play in the lab in the field, namely the deterministic processes of selection, competition, and fitness differences among species (Vellend, 2010). However, the difference in alpha diversity is notable and may affect the degree of microbial community functional redundancy, and consequently the effects of salinity on C mineralization.

5. Conclusions

Salinization is a major issue caused by sea level rise, storms and tides, drought, water management, and waterbody connectivity, which leads to increased ionic strength, alkalinization, and sulfidation, which can result in coastal forest loss, species invasion, yield declines, eutrophication, marsh migration, and changes in microbial communities and biogeochemical cycling (Tully et al., 2019). An outstanding question regarding salinization of coastal wetlands is whether CH_4 emissions will increase or decrease, with important implications for C storage and climate change (Ardón et al., 2018). Our synthesis of microbial and biogeochemical data from multiple studies and methods from multiple geographic locations highlights both some consistencies in the wetland soil microbial communities that regulate CH_4 cycling, as well as some important differences that can qualitatively affect how CH_4 emissions respond to increases in low salinity, including microbial community responses and length of exposure. Key similarities at all sites included a high proportion of Proteobacteria, dominance of hydrogenotrophic methanogens, and general positive relationships between methanogens and the methanogen:methanotroph ratio and CH_4 flux. Some of the main differences were that salinity-CH_4 relationships differed in direction and magnitude, alpha-diversity did not always decrease with salinity, methanogenic genera and responses to salinity differed among sites, and methanotrophic guilds were not always negatively correlated with CH_4 fluxes. Importantly, our results do not support the assumption that seawater intrusion will generally decrease CH_4 emissions from coastal wetlands, at least in the short-term. Further systematic paired sampling of microbial communities and CH_4 flux measurements across a greater number of sites exposed to the same amount of salinity for the same amount of time is needed to directly analyze CH_4/salinity relationship as a response variable, and to be able to make more generalizations about CH_4/salinity relationships in tidal freshwater marshes and other coastal and estuarine wetlands.

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Data Availability Statement

Raw data and analysis scripts for this submission are publicly available on Zenodo https://doi.org/10.5281/zenodo.8250416 (Bueno de Mesquita, 2024). Sequencing data are available on NCBI GenBank with BioProject ID PRJNA1004999 (Bueno de Mesquita, 2023).

Acknowledgments

We acknowledge the JGI sequencing staff for processing the samples and performing the DNA sequencing. We thank all of the team members who helped collect samples in the field and helped run the laboratory incubation experiments. The work (https://doi.org/10.46936/10.25585/60000626) conducted by the U.S. Department of Energy Joint Genome Institute (https://ror.org/04xm1d337), a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. The work at the Alligator site in North Carolina was funded by U.S. National Science Foundation Grants DBI-0805576, DEB-1713592, and EF-1713435 to M.A. The work at the Waccamaw site in South Carolina was funded by a grant from the University of South Carolina Office of Research and Health Sciences Research Funding Program and the US Department of Energy's Office of Science (BER) through the Coastal Center of the National Institute for Climatic Change Research at Tulane University (DOE Grant # DE-FC02-06ER64298) to S.C.N. The work at the Delaware sites was funded by the U.S. National Science Foundation Grant DEB-0919173.

References

Achtnich, C., Bak, F., & Conrad, R. (1995). Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. *Biology and Fertility of Soils*, *19*(1), 65–72. https://doi.org/10.1007/bf00336349

Alam, M., & Jia, Z. (2012). Inhibition of methane oxidation by nitrogenous fertilizers in a paddy soil. *Frontiers in Microbiology*, *3*, 246. https://doi.org/10.3389/fmicb.2012.00246

Al-Haj, A. N., & Fulweiler, R. W. (2020). A synthesis of methane emissions from shallow vegetated coastal ecosystems. *Global Change Biology*, *26*(5), 2988–3005. https://doi.org/10.1111/gcb.15046

Ardón, M., Helton, A. M., & Bernhardt, E. S. (2018). Salinity effects on greenhouse gas emissions from wetland soils are contingent upon hydrologic setting: A microcosm experiment. *Biogeochemistry*, *140*(2), 217–232. https://doi.org/10.1007/s10533-018-0486-2

Ardón, M., Helton, A. M., Scheuerell, M. D., & Bernhardt, E. S. (2017). Fertilizer legacies meet saltwater incursion: Challenges and constraints for coastal plain wetland restoration. *Elementa: Science of the Anthropocene*, *5*. https://doi.org/10.1525/elementa.236

Ardón, M., Morse, J. L., Colman, B. P., & Bernhardt, E. S. (2013). Drought-induced saltwater incursion leads to increased wetland nitrogen export. *Global Change Biology*, *19*(10), 2976–2985. https://doi.org/10.1111/gcb.12287

Ardón, M., Morse, J. L., Doyle, M. W., & Bernhardt, E. S. (2010). The water quality consequences of restoring wetland hydrology to a large agricultural watershed in the southeastern coastal plain. *Ecosystems*, *13*(7), 1060–1078. https://doi.org/10.1007/s10021-010-9374-x

Auguet, J.-C., Barberan, A., & Casamayor, E. O. (2010). Global ecological patterns in uncultured Archaea. *ISME Journal*, *4*(2), 182–190. https://doi.org/10.1038/ismej.2009.109

Bai, Y.-N., Wang, X.-N., Wu, J., Lu, Y.-Z., Fu, L., Zhang, F., et al. (2019). Humic substances as electron acceptors for anaerobic oxidation of methane driven by ANME-2d. *Water Research*, *164*, 114935. https://doi.org/10.1016/j.watres.2019.114935

Baldwin, D. S., Rees, G. N., Mitchell, A. M., Watson, G., & Williams, J. (2006). The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. *Wetlands*, *26*(2), 455–464. https://doi.org/10.1672/0277-5212(2006)26[455:tseoso]2.0.co;2

Barantal, S., Schimann, H., Fromin, N., & Hättenschwiler, S. (2012). Nutrient and carbon limitation on decomposition in an Amazonian moist forest. *Ecosystems*, *15*(7), 1039–1052. https://doi.org/10.1007/s10021-012-9564-9

Bartlett, K. B., Bartlett, D. S., Harriss, R. C., & Sebacher, D. I. (1987). Methane emissions along a salt marsh salinity gradient. *Biogeochemistry*, *4*(3), 183–202. https://doi.org/10.1007/bf02187365

Berga, M., Zha, Y., Székely, A. J., & Langenheder, S. (2017). Functional and compositional stability of bacterial metacommunities in response to salinity changes. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00948

Berrier, D. J., Neubauer, S. C., & Franklin, R. B. (2022). Cooperative microbial interactions mediate community biogeochemical responses to saltwater intrusion in wetland soils. *FEMS Microbiology Ecology*, *98*(3), fiac019. https://doi.org/10.1093/femsec/fiac019

Bodelier, P. L. (2011). Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. *Current Opinion in Environmental Sustainability*, *3*(5), 379–388. https://doi.org/10.1016/j.cosust.2011.06.002

Bodelier, P. L., & Steenbergh, A. K. (2014). Interactions between methane and the nitrogen cycle in light of climate change. *Current Opinion in Environmental Sustainability*, *9–10*, 26–36. https://doi.org/10.1016/j.cosust.2014.07.004

Bodelier, P. L. E., & Laanbroek, H. J. (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, *47*(3), 265–277. https://doi.org/10.1016/s0168-6496(03)00304-0

Bueno de Mesquita, C. P., Hartman, W. H., Ardón, M., & Tringe, S. G. (2024). Disentangling the effects of sulfate and other seawater ions on microbial communities and greenhouse gas emissions in a coastal forested wetland. *ISME Communications*, *4*(1), ycae040. https://doi.org/10.1093/ismeco/ycae040

Bueno de Mesquita, C. P., Schmidt, S. K., & Suding, K. N. (2019). Litter-driven feedbacks influence plant colonization of a high elevation early successional ecosystem. *Plant and Soil*, *444*(1–2), 71–85. https://doi.org/10.1007/s11104-019-04242-3

Bueno de Mesquita, C. P., Wu, D., & Tringe, S. G. (2023). Methyl-based methanogenesis: An ecological and genomic review. *Microbiology and Molecular Biology Reviews*, *0*(1), e00024-22. https://doi.org/10.1128/mmbr.00024-22

Bueno de Mesquita, C. P. (2023). Microbial ecology and site characteristics underlie differences in salinity-methane relationships in coastal wetlands. *PRJNA1004999. NCBI.*

Bueno de Mesquita, C. P. (2024). *JGR resubmission (2.0.0)*. Zenodo. http://doi.org/10.5281/zenodo.8250416

Cai, Z., Shan, Y., & Xu, H. (2007). Effects of nitrogen fertilization on CH_4 emissions from rice fields. *Soil Science & Plant Nutrition*, *53*(4), 353–361. https://doi.org/10.1111/j.1747-0765.2007.00153.x

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Cervantes, F. J., de Bok, F. A. M., Duong-Dac, T., Stams, A. J. M., Lettinga, G., & Field, J. A. (2002). Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. *Environmental Microbiology*, *4*(1), 51–57. https://doi.org/10.1046/j.1462-2920.2002.00258.x

Chamberlain, S. D., Hemes, K. S., Eichelmann, E., Szutu, D. J., Verfaillie, J. G., & Baldocchi, D. D. (2020). Effect of drought-induced salinization on wetland methane emissions, gross ecosystem productivity, and their interactions. *Ecosystems*, *23*(3), 675–688. https://doi.org/10.1007/s10021-019-00430-5

Chambers, L. G., Davis, S. E., Troxler, T., Boyer, J. N., Downey-Wall, A., & Scinto, L. J. (2014). Biogeochemical effects of simulated sea level rise on carbon loss in an Everglades mangrove peat soil. *Hydrobiologia*, *726*(1), 195–211. https://doi.org/10.1007/s10750-013-1764-6

Chambers, L. G., Osborne, T. Z., & Reddy, K. R. (2013). Effect of salinity-altering pulsing events on soil organic carbon loss along an intertidal wetland gradient: A laboratory experiment. *Biogeochemistry*, *115*(1–3), 363–383. https://doi.org/10.1007/s10533-013-9841-5

Chambers, L. G., Reddy, K. R., & Osborne, T. Z. (2011). Short-term response of carbon cycling to salinity pulses in a freshwater wetland. *Soil Science Society of America Journal*, *75*(5), 2000–2007. https://doi.org/10.2136/sssaj2011.0026

Chen, L., Li, C., Feng, Q., Wei, Y., Zheng, H., Zhao, Y., et al. (2017). Shifts in soil microbial metabolic activities and community structures along a salinity gradient of irrigation water in a typical arid region of China. *Science of the Total Environment*, *598*, 64–70. https://doi.org/10.1016/j.scitotenv.2017.04.105

Chen, Y., Dumont, M. G., Neufeld, J. D., Bodrossy, L., Stralis-Pavese, N., McNamara, N. P., et al. (2008). Revealing the uncultivated majority: Combining DNA stable-isotope probing, multiple displacement amplification and metagenomic analyses of uncultivated Methylocystis in acidic peatlands. *Environmental Microbiology*, *10*, 2609–2622. https://doi.org/10.1111/j.1462-2920.2008.01683.x

Coates, J. D., Ellis, D. J., Blunt-Harris, E. L., Gaw, C. V., Roden, E. E., & Lovley, D. R. (1998). Recovery of humic-reducing bacteria from a diversity of environments. *Applied and Environmental Microbiology*, *64*(4), 1504–1509. https://doi.org/10.1128/aem.64.4.1504-1509.1998

Conrad, R. (2007). Microbial ecology of methanogens and methanotrophs. In *Advances in agronomy* (pp. 1–63). Academic Press.

Conrad, R. (2020). Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: A mini review. *Pedosphere*, *30*(1), 25–39. https://doi.org/10.1016/s1002-0160(18)60052-9

Crain, C. M., Silliman, B. R., Bertness, S. L., & Bertness, M. D. (2004). Physical and biotic drivers of plant distribution across estuarine salinity gradients. *Ecology*, *85*(9), 2539–2549. https://doi.org/10.1890/03-0745

Crump, B. C., Hopkinson, C. S., Sogin, M. L., & Hobbie, J. E. (2004). Microbial biogeography along an estuarine salinity gradient: Combined influences of bacterial growth and residence time. *Applied and Environmental Microbiology*, *70*(3), 1494–1505. https://doi.org/10.1128/aem.70.3.1494-1505.2004

Cunha, M. A., Almeida, M. A., & Alcântara, F. (2000). Patterns of ectoenzymatic and heterotrophic bacterial activities along a salinity gradient in a shallow tidal estuary. *Marine Ecology Progress Series*, *204*, 1–12. https://doi.org/10.3354/meps204001

Dalal, R. C., Allen, D. E., Livesley, S. J., & Richards, G. (2008). Magnitude and biophysical regulators of methane emission and consumption in the Australian agricultural, forest, and submerged landscapes: A review. *Plant and Soil*, *309*(1–2), 43–76. https://doi.org/10.1007/s11104-007-9446-7

Dang, C., Morrissey, E. M., Neubauer, S. C., & Franklin, R. B. (2019). Novel microbial community composition and carbon biogeochemistry emerge over time following saltwater intrusion in wetlands. *Global Change Biology*, *25*(2), 549–561. https://doi.org/10.1111/gcb.14486

De Cáceres, M., & Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. *Ecology*, *90*(12), 3566–3574. https://doi.org/10.1890/08-1823.1

Dedysh, S. N., Belova, S. E., Bodelier, P. L. E., Smirnova, K. V., Khmelenina, V. N., Chidthaisong, A., et al. (2007). *Methylocystis heyeri* sp. nov., a novel type II methanotrophic bacterium possessing 'signature' fatty acids of type I methanotrophs. *International Journal of Systematic and Evolutionary Microbiology*, *57*(3), 472–479. https://doi.org/10.1099/ijs.0.64623-0

DeLaune, R. D., Smith, C. J., & Patrick, W. H. (1983). Methane release from Gulf coast wetlands. *Tellus B: Chemical and Physical Meteorology*, *35*(1), 8–15. https://doi.org/10.1111/j.1600-0889.1983.tb00002.x

Dragone, N. B., Diaz, M. A., Hogg, I. D., Lyons, W. B., Jackson, W. A., Wall, D. H., et al. (2021). Exploring the boundaries of microbial habitability in soil. *Journal of Geophysical Research: Biogeosciences*, *126*(6), e2020JG006052. https://doi.org/10.1029/2020jg006052

Dunfield, P., & Knowles, R. (1995). Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a humisol. *Applied and Environmental Microbiology*, *61*(8), 3129–3135. https://doi.org/10.1128/aem.61.8.3129-3135.1995

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Etheridge, D. M., Steele, L. P., Francey, R. J., & Langenfelds, R. L. (1998). Atmospheric methane between 1000 A.D. and present: Evidence of anthropogenic emissions and climatic variability. *Journal of Geophysical Research*, *103*(D13), 15979–15993. https://doi.org/10.1029/98jd00923

Franklin, R. B., Morrissey, E. M., & Morina, J. C. (2017). Changes in abundance and community structure of nitrate-reducing bacteria along a salinity gradient in tidal wetlands. *Pedobiologia*, *60*, 21–26. https://doi.org/10.1016/j.pedobi.2016.12.002

Gavande, P. V., Basak, A., Sen, S., Lepcha, K., Murmu, N., Rai, V., et al. (2021). Functional characterization of thermotolerant microbial consortium for lignocellulolytic enzymes with central role of Firmicutes in rice straw depolymerization. *Scientific Reports*, *11*(1), 3032. https://doi.org/10.1038/s41598-021-82163-x

Georges des Aulnois, M., Roux, P., Caruana, A., Réveillon, D., Briand, E., Hervé, F., et al. (2019). Physiological and metabolic responses of freshwater and brackish-water strains of microcystis aeruginosa acclimated to a salinity gradient: Insight into salt tolerance. *Applied and Environmental Microbiology*, *85*(21). https://doi.org/10.1128/aem.01614-19

Grimsditch, G., Alder, J., Nakamura, T., Kenchington, R., & Tamelander, J. (2013). The blue carbon special edition—Introduction and overview. *Ocean & Coastal Management*, *83*, 1–4. https://doi.org/10.1016/j.ocecoaman.2012.04.020

Guerrero-Cruz, S., Vaksmaa, A., Horn, M. A., Niemann, H., Pijuan, M., & Ho, A. (2021). Methanotrophs: Discoveries, environmental relevance, and a perspective on current and future applications. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.678057

Gunde-Cimerman, N., Plemenitaš, A., & Oren, A. (2018). Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. *FEMS Microbiology Reviews*, *42*(3), 353–375. https://doi.org/10.1093/femsre/fuy009

Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological Reviews*, *60*(2), 439–471. https://doi.org/10.1128/mmbr.60.2.439-471.1996

Hartman, W. H., Bueno de Mesquita, C. P., Theroux, S. M., Morgan-Lang, C., Baldocchi, D. D., & Tringe, S. G. (2024). Multiple microbial guilds mediate soil methane cycling along a wetland salinity gradient. *Msystems*, *9*(1). e00936-23. https://doi.org/10.1128/msystems.00936-23

Helton, A. M., Ardón, M., & Bernhardt, E. S. (2019). Hydrologic context alters greenhouse gas feedbacks of coastal wetland salinization. *Ecosystems*, *22*(5), 1108–1125. https://doi.org/10.1007/s10021-018-0325-2

Hemes, K. S., Chamberlain, S. D., Eichelmann, E., Knox, S. H., & Baldocchi, D. D. (2018). A biogeochemical compromise: The high methane cost of sequestering carbon in restored wetlands. *Geophysical Research Letters*, *45*(12), 6081–6091. https://doi.org/10.1029/2018gl077747

Herbert, E. R., Boon, P., Burgin, A. J., Neubauer, S. C., Franklin, R. B., Ardón, M., et al. (2015). A global perspective on wetland salinization: Ecological consequences of a growing threat to freshwater wetlands. *Ecosphere*, *6*(10), 1–43. https://doi.org/10.1890/es14-00534.1

Herbert, E. R., Schubauer-Berigan, J. P., & Craft, C. B. (2020). Effects of 10 yr of nitrogen and phosphorus fertilization on carbon and nutrient cycling in a tidal freshwater marsh. *Limnology & Oceanography*, *65*(8), 1669–1687. https://doi.org/10.1002/lno.11411

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal*, *5*(10), 1571–1579. https://doi.org/10.1038/ismej.2011.41

Hopple, A. M., Pennington, S. C., Megonigal, J. P., Bailey, V., & Bond-Lamberty, B. (2022). Disturbance legacies regulate coastal forest soil stability to changing salinity and inundation: A soil transplant experiment. *Soil Biology and Biochemistry*, *169*, 108675. https://doi.org/10.1016/j.soilbio.2022.108675

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Hu, M., Peñuelas, J., Sardans, J., Yang, X., Tong, C., Zou, S., & Cao, W. (2020). Shifts in microbial biomass C/N/P stoichiometry and bacterial community composition in subtropical estuarine tidal marshes along a gradient of freshwater–oligohaline water. *Ecosystems*, *23*(6), 1265–1280. https://doi.org/10.1007/s10021-019-00468-5

Huang, J., Zhu, J., Liu, S., Luo, Y., Zhao, R., Guo, F., & Li, B. (2022). Estuarine salinity gradient governs sedimentary bacterial community but not antibiotic resistance gene profile. *Science of the Total Environment*, *806*, 151390. https://doi.org/10.1016/j.scitotenv.2021.151390

Ikenaga, M., Guevara, R., Dean, A. L., Pisani, C., & Boyer, J. N. (2010). Changes in community structure of sediment bacteria along the Florida coastal everglades marsh–mangrove–seagrass salinity gradient. *Microbial Ecology*, *59*(2), 284–295. https://doi.org/10.1007/s00248-009-9572-2

IPCC. (2021). *Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge University Press.

Jackson, C. R., & Vallaire, S. C. (2009). Effects of salinity and nutrients on microbial assemblages in Louisiana wetland sediments. *Wetlands*, *29*(1), 277–287. https://doi.org/10.1672/08-86.1

Juutinen, S., Moore, T. R., Bubier, J. L., Arnkil, S., Humphreys, E., Marincak, B., et al. (2018). Long-term nutrient addition increased CH_4 emission from a bog through direct and indirect effects. *Scientific Reports*, *8*(1), 3838. https://doi.org/10.1038/s41598-018-22210-2

Kausar, H., Sariah, M., Mohd Saud, H., Zahangir Alam, M., & Razi Ismail, M. (2011). Isolation and screening of potential actinobacteria for rapid composting of rice straw. *Biodegradation*, *22*(2), 367–375. https://doi.org/10.1007/s10532-010-9407-3

Kelleway, J. J., Serrano, O., Baldock, J. A., Burgess, R., Cannard, T., Lavery, P. S., et al. (2020). A national approach to greenhouse gas abatement through blue carbon management. *Global Environmental Change*, *63*, 102083. https://doi.org/10.1016/j.gloenvcha.2020.102083

Kelley, C. A., Martens, C. S., & Chanton, J. P. (1990). Variations in sedimentary carbon remineralization rates in the White Oak River estuary, North Carolina. *Limnology & Oceanography*, *35*(2), 372–383. https://doi.org/10.4319/lo.1990.35.2.0372

Ket, W. A., Schubauer-Berigan, J. P., & Craft, C. B. (2011). Effects of five years of nitrogen and phosphorus additions on a Zizaniopsis miliacea tidal freshwater marsh. *Aquatic Botany*, *95*(1), 17–23. https://doi.org/10.1016/j.aquabot.2011.03.003

Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Frontiers in Microbiology*, *6*, 1346. https://doi.org/10.3389/fmicb.2015.01346

Kolde, R. (2019). pheatmap: Pretty heatmaps. R package version 1.0.12. https://CRAN.R-project.org/package=pheatmap(Rpackageversion1.0.12).R

Krauss, K. W., & Whitbeck, J. L. (2012). Soil greenhouse gas fluxes during wetland forest retreat along the lower Savannah River, Georgia (USA). *Wetlands*, *32*(1), 73–81. https://doi.org/10.1007/s13157-011-0246-8

Kristjansson, J. K., & Schönheit, P. (1983). Why do sulfate-reducing bacteria outcompete methanogenic bacteria for substrates? *Oecologia*, *60*(2), 264–266. https://doi.org/10.1007/bf00379530

Kurth, J. M., Op den Camp, H. J. M., & Welte, C. U. (2020). Several ways one goal—Methanogenesis from unconventional substrates. *Applied Microbiology and Biotechnology*, *104*(16), 6839–6854. https://doi.org/10.1007/s00253-020-10724-7

Laas, P., Ugarelli, K., Travieso, R., Stumpf, S., Gaiser, E. E., Kominoski, J. S., & Stingl, U. (2022). Water column microbial communities vary along salinity gradients in the Florida coastal everglades wetlands. *Microorganisms*, *10*(2), 215. https://doi.org/10.3390/microorganisms10020215

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, *75*(15), 5111–5120. https://doi.org/10.1128/aem.00335-09

Leff, J. W. (2022). mctoolsr: Microbial community data analysis tools (0.1.1.9). R. https://github.com/leffj/mctoolsr

Lew, S., Glińska-Lewczuk, K., Burandt, P., Kulesza, K., Kobus, S., & Obolewski, K. (2022). Salinity as a determinant structuring microbial communities in coastal lakes. *International Journal of Environmental Research and Public Health*, *19*(8), 4592. https://doi.org/10.3390/ijerph19084592

Liu, X., Ruecker, A., Song, B., Xing, J., Conner, W. H., & Chow, A. T. (2017). Effects of salinity and wet–dry treatments on C and N dynamics in coastal-forested wetland soils: Implications of sea level rise. *Soil Biology and Biochemistry*, *112*, 56–67. https://doi.org/10.1016/j.soilbio.2017.04.002

Lovley, D. R. (1991). Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews*, *55*(2), 259–287. https://doi.org/10.1128/mr.55.2.259-287.1991

Lovley, D. R., Dwyer, D. F., & Klug, M. J. (1982). Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Applied and Environmental Microbiology*, *43*(6), 1373–1379. https://doi.org/10.1128/aem.43.6.1373-1379.1982

Lovley, D. R., & Klug, M. J. (1983). Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. *Applied and Environmental Microbiology*, *45*(1), 187–192. https://doi.org/10.1128/aem.45.1.187-192.1983

Lozupone, C. A., & Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(27), 11436–11440. https://doi.org/10.1073/pnas.0611525104

Luo, M., Huang, J.-F., Zhu, W.-F., & Tong, C. (2019). Impacts of increasing salinity and inundation on rates and pathways of organic carbon mineralization in tidal wetlands: A review. *Hydrobiologia*, *827*(1), 31–49. https://doi.org/10.1007/s10750-017-3416-8

Luo, Z., Wang, E., & Smith, C. (2015). Fresh carbon input differentially impacts soil carbon decomposition across natural and managed systems. *Ecology*, *96*(10), 2806–2813. https://doi.org/10.1890/14-2228.1

Maltby, J., Sommer, S., Dale, A. W., & Treude, T. (2016). Microbial methanogenesis in the sulfate-reducing zone of surface sediments traversing the Peruvian margin. *Biogeosciences*, *13*(1), 283–299. https://doi.org/10.5194/bg-13-283-2016

Marton, J. M., Herbert, E. R., & Craft, C. B. (2012). Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils. *Wetlands*, *32*(2), 347–357. https://doi.org/10.1007/s13157-012-0270-3

Mcleod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., et al. (2011). A blueprint for blue carbon: Toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. *Frontiers in Ecology and the Environment*, *9*(10), 552–560. https://doi.org/10.1890/110004

Megonigal, J. P., & Neubauer, S. C. (2019). Chapter 19—biogeochemistry of tidal freshwater wetlands. In G. M. E. Perillo, E. Wolanski, D. R. Cahoon, & C. S. Hopkinson (Eds.), *Coastal wetlands* (2nd ed., pp. 641–683). Elsevier.

Mohamed, D. J., & Martiny, J. B. (2011). Patterns of fungal diversity and composition along a salinity gradient. *ISME Journal*, *5*(3), 379–388. https://doi.org/10.1038/ismej.2010.137

Monk, J. M., Charusanti, P., Aziz, R. K., Lerman, J. A., Premyodhin, N., Orth, J. D., et al. (2013). Genome-scale metabolic reconstructions of multiple *Escherichia coli* strains highlight strain-specific adaptations to nutritional environments. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(50), 20338–20343. https://doi.org/10.1073/pnas.1307797110

Morgan-Lang, C., McLaughlin, R., Armstrong, Z., Zhang, G., Chan, K., & Hallam, S. J. (2020). TreeSAPP: The tree-based sensitive and accurate phylogenetic profiler. *Bioinformatics*, *36*(18), 4706–4713. https://doi.org/10.1093/bioinformatics/btaa588

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Morina, J. C., & Franklin, R. B. (2022). Intensity and duration of exposure determine prokaryotic community response to salinization in freshwater wetland soils. *Geoderma*, *428*, 116138. https://doi.org/10.1016/j.geoderma.2022.116138
Morrissey, E. M., Berrier, D. J., Neubauer, S. C., & Franklin, R. B. (2014). Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. *Biogeochemistry*, *117*(2–3), 473–490. https://doi.org/10.1007/s10533-013-9894-5
Morrissey, E. M., & Franklin, R. B. (2015). Resource effects on denitrification are mediated by community composition in tidal freshwater wetlands soils. *Environmental Microbiology*, *17*(5), 1520–1532. https://doi.org/10.1111/1462-2920.12575
Morrissey, E. M., Gillespie, J. L., Morina, J. C., & Franklin, R. B. (2014). Salinity affects microbial activity and soil organic matter content in tidal wetlands. *Global Change Biology*, *20*(4), 1351–1362. https://doi.org/10.1111/gcb.12431
Nellemann, C. (2009). Blue carbon. A UNEP rapid response assessment.
Neubauer, S. C. (2013). Ecosystem responses of a tidal freshwater marsh experiencing saltwater intrusion and altered hydrology. *Estuaries and Coasts*, *36*(3), 491–507. https://doi.org/10.1007/s12237-011-9455-x
Neubauer, S. C., Franklin, R. B., & Berrier, D. J. (2013). Saltwater intrusion into tidal freshwater marshes alters the biogeochemical processing of organic carbon. *Biogeosciences*, *10*(12), 8171–8183. https://doi.org/10.5194/bg-10-8171-2013
Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., et al. (2020). A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nature Communications*, *11*(1), 4717. https://doi.org/10.1038/s41467-020-18560-z
Nobu, M. K., Narihiro, T., Kuroda, K., Mei, R., & Liu, W.-T. (2016). Chasing the elusive Euryarchaeota class WSA2: Genomes reveal a uniquely fastidious methyl-reducing methanogen. *ISME Journal*, *10*, 2478–2487. https://doi.org/10.1038/ismej.2016.33
Nyerges, G., & Stein, L. Y. (2009). Ammonia cometabolism and product inhibition vary considerably among species of methanotrophic bacteria. *FEMS Microbiology Letters*, *297*(1), 131–136. https://doi.org/10.1111/j.1574-6968.2009.01674.x
Nyman, J. A., & DeLaune, R. D. (1991). CO^2emission and soil Eh responses to different hydrological conditions in fresh, brackish, and saline marsh soils. *Limnology & Oceanography*, *36*(7), 1406–1414. https://doi.org/10.4319/lo.1991.36.7.1406
Odum, W. E. (1988). Comparative ecology of tidal freshwater and salt marshes. *Annual Review of Ecology and Systematics*, *19*(1), 147–176. https://doi.org/10.1146/annurev.es.19.110188.001051
Oikawa, P. Y., Jenerette, G. D., Knox, S. H., Sturtevant, C., Verfaillie, J., Dronova, I., et al. (2017). Evaluation of a hierarchy of models reveals importance of substrate limitation for predicting carbon dioxide and methane exchange in restored wetlands. *Journal of Geophysical Research: Biogeosciences*, *122*(1), 145–167. https://doi.org/10.1002/2016jg003438
Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2022). vegan: Community ecology package. R package version, *2*, 5–6. https://CRAN.R-project.org/package=vegan(2.6-4).R
Oremland, R. S., Marsh, L. M., & Polcin, S. (1982). Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. *Nature*, *296*(5853), 143–145. https://doi.org/10.1038/296143a0
Oremland, R. S., & Polcin, S. (1982). Methanogenesis and sulfate reduction: Competitive and noncompetitive substrates in estuarine sediments. *Applied and Environmental Microbiology*, *44*(6), 1270–1276. https://doi.org/10.1128/aem.44.6.1270-1276.1982
Oren, A. (1999). Bioenergetic aspects of halophilism. *Microbiology and Molecular Biology Reviews*, *63*(2), 334–348. https://doi.org/10.1128/mmbr.63.2.334-348.1999
Oren, A. (2013). Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. *Frontiers in Microbiology*, https://doi.org/10.3389/fmicb.2013.00315
Oswald, K., Graf, J. S., Littmann, S., Tienken, D., Brand, A., Wehrli, B., et al. (2017). Crenothrix are major methane consumers in stratified lakes. *ISME Journal*, *11*(9), 2124–2140. https://doi.org/10.1038/ismej.2017.77
Pankratov, T., Dedysh, S., & Zavarzin, G. (2006). The leading role of actinobacteria in aerobic cellulose degradation in Sphagnum peat bogs. *Doklady Biological Sciences: Proceedings of the Academy of Sciences of the USSR, Biological Sciences Sections/Translated from Russian*, *410*(1), 428–430. https://doi.org/10.1134/s0012496606050243
Poffenbarger, H. J., Needelman, B. A., & Megonigal, J. P. (2011). Salinity influence on methane emissions from tidal marshes. *Wetlands*, *31*(5), 831–842. https://doi.org/10.1007/s13157-011-0197-0
Portnoy, J. W., & Giblin, A. E. (1997). Biogeochemical effects of seawater restoration to diked salt marshes. *Ecological Applications*, *7*(3), 1054–1063. https://doi.org/10.2307/2269455
Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, *26*(7), 1641–1650. https://doi.org/10.1093/molbev/msp077
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219
R Core Team. (2023). *R: A language and environment for statistical computing (4.2.3)*. R. R Foundation for Statistical Computing.
Reeburgh, W. S. (2007). Oceanic methane biogeochemistry. *Chemical Reviews*, *107*(20), 486–513. https://doi.org/10.1002/chin.200720267
Rey-Sanchez, C., Bohrer, G., Slater, J., Li, Y.-F., Grau-Andrés, R., Hao, Y., et al. (2019). The ratio of methanogens to methanotrophs and water-level dynamics drive methane transfer velocity in a temperate kettle-hole peat bog. *Biogeosciences*, *16*, 3207–3231. https://doi.org/10.5194/bg-16-3207-2019
Roache, M. C., Bailey, P. C., & Boon, P. I. (2006). Effects of salinity on the decay of the freshwater macrophyte, Triglochin procerum. *Aquatic Botany*, *84*(1), 45–52. https://doi.org/10.1016/j.aquabot.2005.07.014
Rocca, J. D., Simonin, M., Bernhardt, E. S., Washburne, A. D., & Wright, J. P. (2020). Rare microbial taxa emerge when communities collide: Freshwater and marine microbiome responses to experimental mixing. *Ecology*, *101*(3), e02956. https://doi.org/10.1002/ecy.2956
Rosentreter, J. A., Al-Haj, A. N., Fulweiler, R. W., & Williamson, P. (2021). Methane and nitrous oxide emissions complicate coastal blue carbon assessments. *Global Biogeochemical Cycles*, *35*(2), e2020GB006858. https://doi.org/10.1029/2020gb006858
Sadeghi, J., Chaganti, S. R., Shahraki, A. H., & Heath, D. D. (2021). Microbial community and abiotic effects on aquatic bacterial communities in north temperate lakes. *Science of the Total Environment*, *781*, 146771. https://doi.org/10.1016/j.scitotenv.2021.146771
Salazar, G., Ruscheweyh, H.-J., Hildebrand, F., Acinas, S. G., & Sunagawa, S. (2021). mTAGs: Taxonomic profiling using degenerate consensus reference sequences of ribosomal RNA genes. *Bioinformatics*, *38*(1), 270–272. https://doi.org/10.1093/bioinformatics/btab465
Sang, S., Zhang, X., Dai, H., Hu, B. X., Ou, H., & Sun, L. (2018). Diversity and predictive metabolic pathways of the prokaryotic microbial community along a groundwater salinity gradient of the Pearl River Delta, China. *Scientific Reports*, *8*(1), 17317. https://doi.org/10.1038/s41598-018-35350-2
Saunois, M., Stavert, A. R., Poulter, B., Bousquet, P., Canadell, J. G., Jackson, R. B., et al. (2020). The global methane budget 2000–2017. *Earth System Science Data*, *12*(3), 1561–1623. https://doi.org/10.5194/essd-12-1561-2020
Saviozzi, A., Cardelli, R., & Di Puccio, R. (2011). Impact of salinity on soil biological activities: A laboratory experiment. *Communications in Soil Science and Plant Analysis*, *42*(3), 358–367. https://doi.org/10.1080/00103624.2011.542226

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Schimel, J. (2000). Rice, microbes and methane. *Nature*, *403*(6768), 375–377. https://doi.org/10.1038/35000325

Schlesner, H., Jenkins, C., & Staley, J. T. (2006). The phylum verrucomicrobia: A phylogenetically heterogeneous bacterial group. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes: Volume 7: Proteobacteria: Delta, epsilon subclass* (pp. 881–896). Springer.

Schönheit, P., Kristjansson, J. K., & Thauer, R. K. (1982). Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. *Archives of Microbiology*, *132*(3), 285–288. https://doi.org/10.1007/bf00407967

Seo, J., Jang, I., Gebauer, G., & Kang, H. (2014). Abundance of methanogens, methanotrophic bacteria, and denitrifiers in rice paddy soils. *Wetlands*, *34*(2), 213–223. https://doi.org/10.1007/s13157-013-0477-y

Shahbaz, M., Kuzyakov, Y., Sanaullah, M., Heitkamp, F., Zelenev, V., Kumar, A., & Blagodatskaya, E. (2017). Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: Mechanisms and thresholds. *Biology and Fertility of Soils*, *53*(3), 287–301. https://doi.org/10.1007/s00374-016-1174-9

Sloan, W. T., Lunn, M., Woodcock, S., Head, I. M., Nee, S., & Curtis, T. P. (2006). Quantifying the roles of immigration and chance in shaping prokaryote community structure. *Environmental Microbiology*, *8*(4), 732–740. https://doi.org/10.1111/j.1462-2920.2005.00956.x

Smith, C. J., DeLaune, R. D., & Patrick, W. H. (1983). Carbon dioxide emission and carbon accumulation in coastal wetlands. *Estuarine, Coastal and Shelf Science*, *17*(1), 21–29. https://doi.org/10.1016/0272-7714(83)90042-2

Stegen, J. C., Lin, X., Konopka, A. E., & Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME Journal*, *6*(9), 1653–1664. https://doi.org/10.1038/ismej.2012.22

Sutton-Grier, A. E., Keller, J. K., Koch, R., Gilmour, C., & Megonigal, J. P. (2011). Electron donors and acceptors influence anaerobic soil organic matter mineralization in tidal marshes. *Soil Biology and Biochemistry*, *43*(7), 1576–1583. https://doi.org/10.1016/j.soilbio.2011.04.008

Takeuchi, M., Katayama, T., Yamagishi, T., Hanada, S., Tamaki, H., Kamagata, Y., et al. (2014). *Methyloceanibacter caenitepidi* gen. nov., sp. nov., a facultatively methylotrophic bacterium isolated from marine sediments near a hydrothermal vent. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_2), 462–468. https://doi.org/10.1099/ijs.0.053397-0

Thelaus, J., Lundmark, E., Lindgren, P., Sjödin, A., & Forsman, M. (2018). *Galleria mellonella* reveals niche differences between highly pathogenic and closely related strains of *Francisella* spp. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00188

Tremblay, J., Singh, K., Fern, A., Kirton, E. S., He, S., Woyke, T., et al. (2015). Primer and platform effects on 16S rRNA tag sequencing. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00771

Tully, K., Gedan, K., Epanchin-Niell, R., Strong, A., Bernhardt, E. S., BenDor, T., et al. (2019). The invisible flood: The chemistry, ecology, and social implications of coastal saltwater intrusion. *BioScience*, *69*(5), 368–378. https://doi.org/10.1093/biosci/biz027

Ury, E. A., Wright, J. P., Ardón, M., & Bernhardt, E. S. (2022). Saltwater intrusion in context: Soil factors regulate impacts of salinity on soil carbon cycling. *Biogeochemistry*, *157*(2), 215–226. https://doi.org/10.1007/s10533-021-00869-6

Valach, A. C., Kasak, K., Hemes, K. S., Szutu, D., Verfaillie, J., & Baldocchi, D. D. (2021). Carbon flux trajectories and site conditions from restored impounded marshes in the Sacramento-San Joaquin Delta. In *Wetland carbon and environmental management* (1st ed., pp. 247–271). John Wiley & Sons, Inc.

Valenzuela, E. I., & Cervantes, F. J. (2021). The role of humic substances in mitigating greenhouse gases emissions: Current knowledge and research gaps. *Science of the Total Environment*, *750*, 141677. https://doi.org/10.1016/j.scitotenv.2020.141677

Vander Vorste, R., Timpano, A. J., Cappellin, C., Badgley, B. D., Zipper, C. E., & Schoenholtz, S. H. (2019). Microbial and macroinvertebrate communities, but not leaf decomposition, change along a mining-induced salinity gradient. *Freshwater Biology*, *64*(4), 671–684. https://doi.org/10.1111/fwb.13253

Van Horn, D. J., Okie, J. G., Buelow, H. N., Gooseff, M. N., Barrett, J. E., & Takacs-Vesbach, C. D. (2014). Soil microbial responses to increased moisture and organic resources along a salinity gradient in a polar desert. *Applied and Environmental Microbiology*, *80*(10), 3034–3043. https://doi.org/10.1128/aem.03414-13

Van Kessel, J., & Russell, J. B. (1996). The effect of pH on ruminal methanogenesis. *FEMS Microbiology Ecology*, *20*(4), 205–210. https://doi.org/10.1016/0168-6496(96)00030-x

Vellend, M. (2010). Conceptual synthesis in community ecology. *The Quarterly Review of Biology*, *85*(2), 183–206. https://doi.org/10.1086/652373

Walse, C., Berg, B., & Sverdrup, H. (1998). Review and synthesis of experimental data on organic matter decomposition with respect to the effect of temperature, moisture, and acidity. *Environmental Reviews*, *6*(1), 25–40. https://doi.org/10.1139/a98-001

Wang, C., Tong, C., Chambers, L. G., & Liu, X. (2017). Identifying the salinity thresholds that impact greenhouse gas production in subtropical tidal freshwater marsh soils. *Wetlands*, *37*(3), 559–571. https://doi.org/10.1007/s13157-017-0890-8

Wang, J., Yang, D., Zhang, Y., Shen, J., van der Gast, C., Hahn, M. W., & Wu, Q. (2011). Do patterns of bacterial diversity along salinity gradients differ from those observed for macroorganisms? *PLoS One*, *6*(11), e27597. https://doi.org/10.1371/journal.pone.0027597

Weston, N. B., Dixon, R. E., & Joye, S. B. (2006). Ramifications of increased salinity in tidal freshwater sediments: Geochemistry and microbial pathways of organic matter mineralization. *Journal of Geophysical Research*, *111*(G1), G01009. https://doi.org/10.1029/2005jg000071

Weston, N. B., Giblin, A. E., Banta, G. T., Hopkinson, C. S., & Tucker, J. (2010). The effects of varying salinity on ammonium exchange in estuarine sediments of the Parker River, Massachusetts. *Estuaries and Coasts*, *33*(4), 985–1003. https://doi.org/10.1007/s12237-010-9282-5

Weston, N. B., Neubauer, S. C., Velinsky, D. J., & Vile, M. A. (2014). Net ecosystem carbon exchange and the greenhouse gas balance of tidal marshes along an estuarine salinity gradient. *Biogeochemistry*, *120*(1–3), 163–189. https://doi.org/10.1007/s10533-014-9989-7

Weston, N. B., Vile, M. A., Neubauer, S. C., & Velinsky, D. J. (2011). Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. *Biogeochemistry*, *102*(1–3), 135–151. https://doi.org/10.1007/s10533-010-9427-4

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. Retrieved from https://ggplot2.tidyverse.org

Wilson, B. J., Mortazavi, B., & Kiene, R. P. (2015). Spatial and temporal variability in carbon dioxide and methane exchange at three coastal marshes along a salinity gradient in a northern Gulf of Mexico estuary. *Biogeochemistry*, *123*(3), 329–347. https://doi.org/10.1007/s10533-015-0085-4

Xu, L., Zhuang, G.-C., Montgomery, A., Liang, Q., Joye, S. B., & Wang, F. (2021). Methyl-compounds driven benthic carbon cycling in the sulfate-reducing sediments of South China Sea. *Environmental Microbiology*, *23*(2), 641–651. https://doi.org/10.1111/1462-2920.15110

Yan, W., Zhong, Y., Zhu, G., Liu, W., & Shangguan, Z. (2020). Nutrient limitation of litter decomposition with long-term secondary succession: Evidence from controlled laboratory experiments. *Journal of Soils and Sediments*, *20*(4), 1858–1868. https://doi.org/10.1007/s11368-019-02523-z

Zervoudaki, S., Nielsen, T. G., & Carstensen, J. (2009). Seasonal succession and composition of the zooplankton community along an eutrophication and salinity gradient exemplified by Danish waters. *Journal of Plankton Research*, *31*(12), 1475–1492. https://doi.org/10.1093/plankt/fbp084

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Zhang, G., Bai, J., Tebbe, C. C., Zhao, Q., Jia, J., Wang, W., et al. (2021). Salinity controls soil microbial community structure and function in coastal estuarine wetlands. *Environmental Microbiology*, *23*(2), 1020–1037. https://doi.org/10.1111/1462-2920.15281

Zhang, Y., Cui, M., Duan, J., Zhuang, X., Zhuang, G., & Ma, A. (2019). Abundance, rather than composition, of methane-cycling microbes mainly affects methane emissions from different vegetation soils in the Zoige alpine wetland. *Microbiologyopen*, *8*(4), e00699. https://doi.org/10.1002/mbo3.699

Zhao, Q., Bai, J., Gao, Y., Zhao, H., Zhang, G., & Cui, B. (2020). Shifts in the soil bacterial community along a salinity gradient in the Yellow River Delta. *Land Degradation & Development*, *31*(16), 2255–2267. https://doi.org/10.1002/ldr.3594

Zhao, X., Meng, T., Jin, S., Ren, K., Cai, Z., Cai, B., & Li, S. (2023). The salinity survival strategy of *Chenopodium quinoa*: Investigating microbial community shifts and nitrogen cycling in saline soils. *Microorganisms*, *11*(12), 2829. https://doi.org/10.3390/microorganisms11122829

Zheng, W., Xue, D., Li, X., Deng, Y., Rui, J., Feng, K., & Wang, Z. (2017). The responses and adaptations of microbial communities to salinity in farmland soils: A molecular ecological network analysis. *Applied Soil Ecology*, *120*, 239–246. https://doi.org/10.1016/j.apsoil.2017.08.019

Zhou, J., Theroux, S. M., Bueno de Mesquita, C. P., Hartman, W. H., & Tringe, S. G. (2021). Microbial drivers of methane emissions from unrestored industrial salt ponds. *ISME Journal*, *16*(1), 284–295. https://doi.org/10.1038/s41396-021-01067-w

Zhou, M., Butterbach-Bahl, K., Vereecken, H., & Brüggemann, N. (2017). A meta-analysis of soil salinization effects on nitrogen pools, cycles and fluxes in coastal ecosystems. *Global Change Biology*, *23*(3), 1338–1352. https://doi.org/10.1111/gcb.13430

21698961, 2024, 6, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2024JG008133, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License