Science of the Total Environment 858 (2023) 159865

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Year-around survey and manipulation experiments reveal differential sensitivities of soil prokaryotic and fungal communities to saltwater intrusion in Florida Everglades wetlands

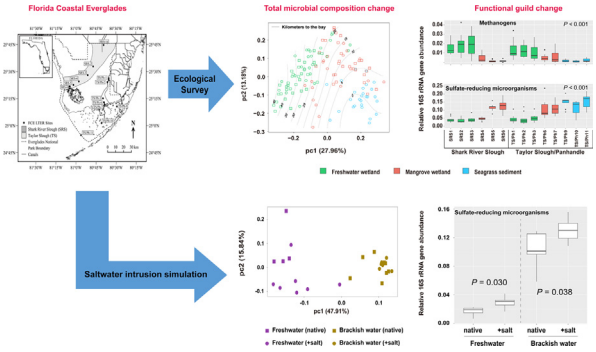
Jun Zhao [a], Seemanti Chakrabarti [a], Randolph Chambers [b], Pamela Weisenhorn [c], Rafael Travieso [d], Sandro Stumpf [d], Emily Standen [d], Henry Briceno [e], Tiffany Troxler [f], Evelyn Gaiser [e], John Kominoski [e], Braham Dhillon [g], Willm Martens-Habbena [a,*]

[a] Fort Lauderdale Research and Education Center and Department of Microbiology & Cell Science, University of Florida, Davie, FL, USA
[b] College of William and Mary, W.M. Keck Environmental Field Laboratory, P.O. Box 8795, Williamsburg, VA, USA
[c] Argonne National Laboratory, Lemont, IL, USA
[d] Institute of Environment, Florida International University, Miami, FL, USA
[e] Department of Biological Sciences and Institute of Environment, Florida International University, Miami, FL, USA
[f] Department of Earth and Environment and Sea Level Solutions Center in the Institute of Environment, Florida International University, Miami, FL, USA
[g] Fort Lauderdale Research and Education Center and Department of Plant Pathology, University of Florida, Davie, FL, USA

HIGHLIGHTS

- Saltwater intrusion threatens coastal freshwater ecosystems worldwide
- A two-year time series wetland soils and experimental simulation was used to investigate microbial response to saltwater intrusion
- Bacterial, archaeal, and fungal communities were analyzed via 16S rRNA- and fungal ITS genes.
- Methanogens and sulfate reducers respond most sensitively to saltwater intrusion
- Sulfate availability can restructure interactions between methanogens and sulfate reducers already at low salinities.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Abasiofiok Mark Ibekwe

Keywords:
Prokaryotic and eukaryotic microorganisms
Spatial and temporal variations
Saltwater intrusion
Experimental manipulation
Florida Everglades
Next-generation sequencing

ABSTRACT

Global sea-level rise is transforming coastal ecosystems, especially freshwater wetlands, in part due to increased episodic or chronic saltwater exposure, leading to shifts in biogeochemistry, plant- and microbial communities, as well as ecological services. Yet, it is still difficult to predict how soil microbial communities respond to the saltwater exposure because of poorly understood microbial sensitivity within complex wetland soil microbial communities, as well as the high spatial and temporal heterogeneity of wetland soils and saltwater exposure. To address this, we first conducted a two-year survey of microbial community structure and bottom water chemistry in submerged surface soils from 14 wetland sites across the Florida Everglades. We identified ecosystem-specific microbial biomarker taxa primarily associated with variation in salinity. Bacterial, archaeal and fungal community composition differed between freshwater, mangrove, and marine seagrass meadow sites, irrespective of soil type or season. Especially, methanogens, putative denitrifying methanotrophs and sulfate reducers shifted in relative abundance and/or composition between wetland types. Methanogens and putative denitrifying methanotrophs declined in relative abundance from freshwater

* Corresponding author.
E-mail address: w.martenshabbena@ufl.edu (W. Martens-Habbena).

http://dx.doi.org/10.1016/j.scitotenv.2022.159865
Received 29 August 2022; Received in revised form 26 October 2022; Accepted 27 October 2022
Available online 30 October 2022

to marine wetlands, whereas sulfate reducers showed the opposite trend. A four-year experimental simulation of saltwater intrusion in a pristine freshwater site and a previously saltwater-impacted site corroborated the highest sensitivity and relative increase of sulfate reducers, as well as taxon-specific sensitivity of methanogens, in response to continuously pulsing of saltwater treatment. Collectively, these results suggest that besides increased salinity, saltwater-mediated increased sulfate availability leads to displacement of methanogens by sulfate reducers even at low or temporal salt exposure. These changes of microbial composition could affect organic matter degradation pathways in coastal freshwater wetlands exposed to sea-level rise, with potential consequences, such as loss of stored soil organic carbon.

1. Introduction

Coastal wetlands represent some of the most productive habitats on Earth (Barbier et al., 2011). They consist of various ecosystem types including freshwater marshes, mangrove forests, tidal salt marshes, and seagrass meadows (Hopkinson et al., 2019). These wetlands support great biodiversity including prokaryotic and eukaryotic microorganisms, which carry out key biogeochemical processes. Coastal wetland soils and sediments also serve as important sinks of CO_2 due to constrained organic matter decomposition under anaerobic conditions (Chmura et al., 2003; Osland et al., 2018). However, they are often net sources of CH_4 and N_2O (Rosentreter et al., 2021; Tan et al., 2020). Many coastal wetland systems are restructured by global change. As a consequence of sea-level rise, increasing saltwater intrusions transform the most exposed parts of freshwater wetlands into mangroves or salt marshes, causing ghost forests and open water areas to occur due to mangrove migration and peat collapse, respectively (Chambers et al., 2019; Church and White, 2011; Craft et al., 2009; Spivak et al., 2019). Salinization affects chemistry and biology of the wetlands, as well as the corresponding biogeochemical processes (Herbert et al., 2015). To predict the impact of such conversion on ecosystem services in coastal wetlands, it is important to gain a comprehensive understanding of the microbial biodiversity within and across different habitat types in spatially and temporal heterogenous wetland soils (Larkin, 2018).

Differences in water chemistry are often closely associated with spatial patterns of microbe-driven biogeochemistry in coastal wetlands. The salinity gradient is one of the characteristic features of coastal wetland ecosystems, and depending on the level of saltwater influence, has been recognized as a critical parameter determining microbial community structure and activity in such environments (Chi et al., 2021b; Ikenaga et al., 2010; Li et al., 2019; Li et al., 2018; Morrissey et al., 2014; Zhang et al., 2021). For instance, salinity is strongly negatively correlated to methane emissions from coastal wetlands, with the lowest emission potential occurring in polyhaline marshes (salinity >18 practical salinity units, psu) whereas oligohaline (0.5–5 psu) or freshwater marshes show the highest and most variable methane emissions (Poffenbarger et al., 2011). Saltwater intrusions also alter soil porewater solute concentrations (Lu et al., 2015; Weissman and Tully, 2020; Wilson et al., 2019) and carbon, nitrogen and sulfur transformations (Liu et al., 2017; Rath and Rousk, 2015; Schoepfer et al., 2014; Zhou et al., 2017). In addition, spatial variation in salinity can lead to plant zonation, affecting primary production, dissolved organic matter (DOM) dynamics, soil light availability, nutrient cycling and microbial growth (Chi et al., 2021a; Logozzo et al., 2021; Osland et al., 2018; Shelton et al., 2021; Spivak et al., 2019). However, the scale at which all the above factors impact microbial communities across or within ecosystems is still not very well understood.

Spatial and temporal heterogeneity in coastal wetlands is particularly high in tropical or subtropical climates due to seasonality of temperature, precipitation and tidal fluctuation. Subtropical and tropical wetlands therefore provide good models to study the effect of variations in water level, redox potential and nutrient concentrations on microbial communities. Indeed, previous studies have found that these variations sometimes affects soil microbial composition (Cheung et al., 2018; Maietta et al., 2020) and related ecosystem functions, such as soil respiration, methanogenesis and denitrification (Cao et al., 2020; Chen et al., 2018; Han et al., 2018; Welti et al., 2016). However, the effects of these variations can complicate the prediction of directional changes in microbial community assemblages associated with sea-level rise. Thus, spatial and temporal scales at which microbial communities fluctuate seasonally in different wetlands must be understood in order to pinpoint changes related to sea-level rise.

The Florida Everglades is the largest contiguous wetland system in the continental United States, and consists of freshwater marshes, estuarine mangroves and coastal seagrass estuary ecosystems located in tropical region of South Florida. The Everglades represents a great model system to examine the gradients of microbial communities within and across different wetland ecosystem types and their response to sea-level rise at comparatively large spatial scales. In this study we investigated the structure and diversity of prokaryotic and fungal communities along two main sloughs within the Everglades National Park, and compositional change of prokaryotes following a four-year in situ experimental simulation of saltwater intrusion. By integrating sequencing data and environmental parameters from seasonal time series and manipulation experiments, we investigated: (i) compositional shifts of overall soil and sediment prokaryotic and fungal microbial communities along the transects within and between different wetland types, including biogeochemically important functional guilds; (ii) The impact of temporal heterogeneity and soil depth on the structure of microbial communities, and (iii) sensitivity of freshwater and brackish water soil microbial communities to experimental saltwater intrusion. Our study represents the first high-resolution spatial and temporal analysis of soil microbial communities across the Florida Everglades and will provide a framework for understanding the general stability and susceptibility of soil microbial communities exposed to sea-level rise-mediated saltwater intrusion.

2. Material and methods

2.1. Site description and soil collection

The Florida Everglades are located in Southwest Florida. Soil and sediment samples were collected as part of the Florida Coastal Everglades (FCE) Long Term Ecological Research (LTER) program from the 14 main long-term monitoring sites along the two major water flow paths of the Everglades, Shark River Slough (SRS) and Taylor Slough/Panhandle (TS/Ph). SRS sites are characterized by peat-based soil, while TS/Ph soils have lower organic matter content and are marl-based. The sampling sites represent three major ecosystem types, i.e. freshwater marshes (SRS1–SRS3 and TS/Ph1–TS/Ph3, respectively), mangrove forests (SRS4–SRS6 and TS/Ph 6–TS/Ph7) and the seagrass meadows located in Florida Bay (TS/Ph9–TS/Ph11). More detailed descriptions of the sampling locations and site metadata can be found at https://fcelter.fiu.edu/ and previous studies (Chen et al., 2013; Childers et al., 2006).

Bulk surface soil samples of each site (~ 0–10 cm) were collected from February 2019 to October 2020 to cover dry and wet seasons (Fig. S1 for detailed sample collection time). Samples were immediately placed on ice and transported to laboratory. The samples were stored at −20 °C until further molecular biological analysis. In addition to bulk soil samples, three replicate soil cores of ~90 mm depth (TS7, ~70 mm) were collected from each site in August 2020, to assess vertical gradients of microbial communities within the soil or sediment column. Soil cores were closed with rubber stoppers, transported back to the laboratory on ice, and immediately sliced into 9-mm sections, resulting in a total of 8–10 soil section samples for each

core. Each sliced soil section was well mixed to homogenize, and 4–5 representative depths (0–9 mm, 18–27 mm, 36–45 mm, 54–63 mm and 72–81 mm) from each soil core were selected for molecular biological analysis.

2.2. Chemical analyses

Due to the relatively small size of soil cores for molecular biological analyses, a bottom water sample was collected at each site for chemical analyses. Water samples were filtered through a Whatman GF/F filter for determination of dissolved organic carbon (DOC), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), soluble reactive phosphorus (SRP) concentrations and salinity. The unfiltered samples were used for determination of total nitrogen (TN) and total phosphorus (TP) contents. DOC was quantified on a Shimadzu TOC Analyzer and other dissolved nutrients were measured following standard rapid flow analyzer (RFA) protocols. TN was measured with an Antec TN analyzer and TP was analyzed following a modified procedure (Solorzano and Sharp, 1980). Salinity was determined using a YSI conductivity meter. All chemical properties are reported in Table S1, and additional information (e.g., chemistry data before year 2019) can be found in FCE datasets (https://fce-lter.fiu.edu/data/core/).

2.3. DNA extraction and amplicon sequencing

Soil samples were homogenized using sterile mortar and pestle before total DNA extraction using DNeasy PowerSoil Kit (QIAGEN) following manufacturer's instruction including bead-beating twice for 30 s at 4.5 m/s in a FastPrep-24 bead beater (MP Biomedical, Irvine, CA). DNA was dissolved in 50 μL molecular biology grade water and stored at −20 °C before use. DNA concentrations were normalized to ~10 ng μL^{-1} and the 16S rRNA- and ITS genes were amplified using primer pair 515f-926r (Parada et al., 2016; Quince et al., 2011) and ITS1f-ITS2 (Gardes and Bruns, 1993; White et al., 1990), respectively, fused with specific adaptors following Earth Microbiome protocol (Thompson et al., 2017). The PCR amplification, library construction (2 × 250 bp) and sequencing of the amplicons were performed on an Illumina NovaSeq instrument at Novogene, Sacramento, CA following standard procedures. Demultiplexed sequencing data were deposited at NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA804243, PRJNA804246 and PRJNA804228 for 16S rRNA genes of bulk surface soil samples, ITS genes of bulk surface soil samples and 16S rRNA genes of depth gradient soil samples, respectively.

2.4. Sequencing data processing

The raw sequences were processed using QIIME2 (Caporaso et al., 2010), including quality filtering, denoising, pair-end read merging and dereplication by DADA2 to generate amplicon sequence variants (ASVs) (Callahan et al., 2016). This resulted in 63,265 ± 5720 and 66,654 ± 27,714 high-quality reads of 16S rRNA and ITS gene sequences per sample after quality filtering (Q score ≥ 30), respectively. ASVs of 16S rRNA and ITS genes were taxonomically classified against SILVA database release 138 for prokaryotes and UNITE ITS Reference Sequences V8.2 for fungi, respectively, using the scikit-learn classifier (Pedregosa et al., 2011) implemented in QIIME2. Alpha and beta-diversity of were calculated using "qiime diversity core-metrics-phylogenetic" plugin, after random subsampling of 35,000 and 10,000 reads per sample for 16S rRNA and ITS genes, respectively. For prokaryotes, species/genera belonging to functional groups including methanogens, denitrifying methanotrophs (also known as NC10 phylum), sulfate reducers and nitrifiers were manually identified in the dataset and combined for statistical analysis. Other functional groups potentially relevant to important wetland ecosystem services, including conventional aerobic methanotrophs and anaerobic ammonia oxidizers (anammox), were only detected in a small subset of samples, and at very low relative frequencies, and thus were not included in further statistical analyses.

2.5. Saltwater intrusion experiment

Experimental pulsed addition of artificial saltwater was conducted in two marsh sites along the southeastern boundary of SRS within the Everglades National Park, including one freshwater marsh site (25 26′ 6.11" N, 80 46′ 50.78" W; target porewater salinity of 5 psu) with no history of saltwater intrusion, and one brackish site with previous experience of saltwater intrusion (25 13′ 13.38" N, 80 50′ 36.66′ W; target porewater salinity of 20 psu). The saltwater addition started in October 2014 on a monthly basis and continued through October 2018. A detailed description of the experimental setup, soil physiochemistry, and biological properties were reported previously (Servais et al., 2020; Wilson et al., 2018). Soil samples from saltwater-exposed soils and unmanipulated native soils from both sites were collected in September 2018 and DNA extraction was conducted as described above. DNA extraction, PCR amplification using primer pair 515F-806R (Apprill et al., 2015; Parada et al., 2016), library preparation (2 × 150 bp) and sequencing on an Illumina MiSeq instrument was conducted at Argonne National Laboratory, Chicago, Illinois. The sequence processing and statistical analysis followed procedures as described above and resulted in 64,607 ± 12,708 high-quality reads of 16S rRNA genes. Samples with <12,000 reads were removed from further analysis, leaving between 4 and 8 biological replicates of each treatment for downstream analyses. Random subsampling of 12,000 reads was conducted for diversity index calculation. Demultiplexed sequencing data were deposited into the NCBI SRA under BioProject accession number PRJNA804545.

2.6. Statistical analyses

Principal Coordinates Analysis (PCoA) of weighted UniFrac distance matrices of 16S rRNA and ITS ASVs were performed using the vegan package version 2.5–7 (Dixon, 2003) in R version 4.1.1 (R Core Team, 2020). Differences in microbial community structure were evaluated by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations between sample groups, including ecosystem, site, season, sediment depth, and their interactions as fixed factors, and $p < 0.05$ indicating significant differences. To characterize the correlation of environmental variables and microbial community composition, the 'envfit' function of the vegan package was used to test the significance of overlaying water chemical properties (as vector parameters, i.e., salinity, concentrations of DOC, TN, NH_4^+, NO_2^-, NO_3^-, TP, SRP) and site descriptive characteristics (as factor parameters, i.e., dry or wet seasons, different ecosystem types and estuary channels) for the Principal Coordinates Analysis (PCoA) ordinations. This model appeared acceptable because no vertical chemical gradients were observed in the top 10 cm soil samples in long-term dataset from previous years (Chambers and Pederson, 2006), as well as in our August 2020 samples. To illustrate the relationship between community compositions and site distances to the estuarine coastal line, isolines were fitted to PCoA using generalized additive models with the vegan function 'ordisurf'. Differences at $p < 0.05$ were considered statistically significant. To assess the degree of seasonal variations in microbial community compositions from each site, within-site similarities of bacterial communities were obtained by calculating weighted UniFrac distances of samples to the respective site centroid using the function 'betadisper' (Anderson, 2006). Data were visualized using the phyloseq and ggplot2 packages in R (McMurdie and Holmes, 2013). Linear Discriminant Effect Size analysis (LEfSe) was performed to identify biomarker taxa (prokaryotes at order level and fungi at genus level) from each of the three main ecosystems (i.e., freshwater wetlands, mangrove forests and seagrass meadows) or at different depths of the sediment cores (i.e., top and 10-cm depth sections of the collected sediment soil cores) in Galaxy (Segata et al., 2011). After comparison of different distance matrices, order-level bacterial and archaeal taxa were used for LEfSe analysis for balance between high level and low level taxonomic changes to best capture the diverse biomarkers in different wetlands. Differential abundances of taxa between different ecosystems or sediment depths were identified by Kruskal-Wallis nonparametric rank-sum test, following Linear Discriminant Analysis (LDA)

to determine the effect size. Significance was determined at $\alpha < 0.05$ and LDA score > 3.0 (for 16S rRNA gene) or > 2.0 (for ITS gene).

3. Results

3.1. Soil prokaryotic and fungal community structure in main wetland types

Prokaryotic and fungal communities were analyzed in bulk soil samples collected seasonally from February 2019 to October 2020 by amplicon-based sequencing of 16S rRNA- and ITS genes, respectively. Prokaryotic and fungal composition differed distinctly between freshwater, mangrove and seagrass sediment sites regardless of sampling time (PERMANOVA, $p < 0.001$ for both prokaryotes and fungi, Fig. 1). A spatial shift in community compositions was strongly associated with site horizontal geographic distances to the coastal bay line ($r^2 = 0.41$ for prokaryotes and $r^2 = 0.35$ for fungi, both $p < 0.001$), as visualized by a generalized additive model fitted to the PCoA. As expected, among all tested chemical properties, salinity had the strongest correlation with the shift in both prokaryotic and fungal community composition across different ecosystems ($r^2 = 0.41$, $p < 0.001$ for prokaryotes and $r^2 = 0.23$, $p = 0.002$ for fungi), corroborating recent findings for water column microbial communities in the FCE (Laas et al., 2022). Concentrations of soluble reactive phosphorus (SRP, $r^2 = 0.24$, $p < 0.001$) and dissolved organic carbon (DOC, $r^2 = 0.15$, $p = 0.006$) were also strongly correlated with overall prokaryotic, but not fungal community composition across all wetland soils (Table S2).

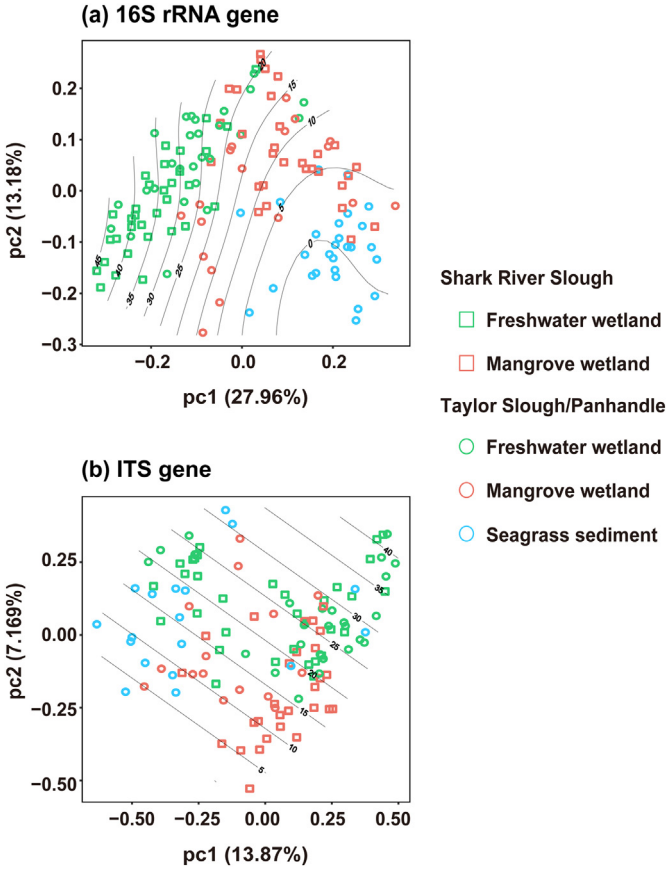


Fig. 1. PCoA of weighted UniFrac distances of microbial communities based on 16S rRNA genes (a) and ITS genes (b) from 14 wetland sites in Shark River Slough and Taylor Slough/Panhandle from February 2019 to October 2020, representing three distinct wetland ecosystem types (freshwater wetland, mangrove forest and seagrass meadow). Effect of distance from the coastal bay line (kilometer, indicated by grey line) on community composition is visualized using a generalized additive model.

Salinity did not change between different sites of the same wetland type (Table S1), suggesting other environmental variables might contribute to structuring the microbial assemblage within each ecosystem type. Therefore, we assessed the importance of environmental parameters influencing microbial compositions in soils of each wetland ecosystem type. The results indeed showed that salinity was similar and did not correlate to the microbial composition variations between different sites from the same ecosystem type ($p > 0.1$ in all three ecosystem types, Tables S3 and S4). However, changes in DOC ($r^2 = 0.34, p = 0.002$) and SRP ($r^2 = 0.21, p = 0.026$) were significant and associated with shift in prokaryotic compositions between different freshwater marsh soils, while ammonium concentrations ($r^2 = 0.19, p = 0.04$) were correlated with variation in prokaryotic assemblages only in mangrove soils (Table S3). Changes in DOC concentration ($r^2 = 0.19, p = 0.042$) was correlated with shifts in fungal compositions in freshwater sites, whereas TP ($r^2 = 0.27, p = 0.023$) and nitrate plus nitrite ($r^2 = 0.22, p = 0.040$) concentrations influenced the fungal compositions in mangrove sites (Table S4). However, none of the tested environmental parameters correlated significantly with prokaryotic or fungal compositional variations among seagrass meadow sediments (Tables S3 and S4).

Biomarker taxa specifically enriched in each ecosystem type (i.e., freshwater marsh, mangrove wetland or seagrass sediments) and contributing significantly to the differences in microbial community composition between the three ecosystem types were identified using LEfSe analysis. A total of 28, 5, 27 prokaryotic biomarker taxa at the order level (Fig. 2a) and 7, 11, 3 fungal biomarkers at genus level (Fig. 2b) were identified from the freshwater marsh, mangrove wetland and seagrass bed sediments, respectively (see Tables S5 and S6 for full list of prokaryotic and fungal biomarker taxa and their relative abundances). Interestingly, mangrove wetlands had the lowest number of prokaryotic biomarkers but the highest number of fungal biomarker taxa. We then characterized the common biomarkers (average relative abundance >1 % for prokaryotes and 0.5 % for fungi, see Fig. S1 and S2 for full list of common prokaryotic and fungal taxa, respectively) in each of the three ecosystems. For Archaea, the orders *Bathyarchaeia* (mean ± SE = 8.0 % ± 0.8 % in freshwater wetlands) and *Lokiarchaeia* (1.7 % ± 0.4 % in seagrass sediments) represented the most commonly detected biomarker taxa in freshwater wetland and seagrass sediments, respectively. It is worth mentioning that three less frequently detected methanogenic archaeal orders, i.e., *Methanobacteriales*, *Methanomicrobiales*, *Methanosarciniales* (with average relative abundance of 0.3 %–0.5 %), were also biomarkers in freshwater wetland sediments. In comparison, no archaeal order was particularly enriched and identified as biomarker taxon in mangrove wetland soils. For bacterial communities, *Burkholderiales* (6.1 % ± 0.4 %), *Vicinamibacterales* (1.2 % ± 0.1 %) and an unclassified *Desulfobacterota* order (1.1 % ± 0.1 %) were the only three common biomarkers at order level in freshwater sediments. It is noteworthy that the order of *Methylomirabilales* (0.4 % ± 0.1 %, previously assigned to NC10 phylum), which contains denitrifying methanotrophs, was also a biomarker in freshwater wetlands. Seagrass sediments contained 12 common bacterial biomarkers at order level, among which two orders, i.e., *Desulfobacterales* (8.2 % ± 0.5 %) and *Desulfobulbales* (3.8 % ± 0.5 %), represented sulfate reducers. Mangrove wetlands had only one common bacterial biomarker at order level, i.e., *Ardenticatenales* (1.6 % ± 0.1 %). For fungal communities, all biomarker genera in freshwater wetlands were from phylum *Ascomycota,* including *Echria* (12.0 % ± 3.1 %), *Acremonium* (4.2 % ± 1.1 %), *Paraphaeosphaeria* (1.6 % ± 0.3 %) and *Kohlmeyeriopsis* (1.4 % ± 0.5 %) as the common biomarker taxa. The *Lulworthia* (2.4 % ± 1.6 %) and an unclassified *Sporocadaceae* genera (2.6 % ± 0.8 %) from phylum *Ascomycota* represented the common biomarker taxa in the mangrove wetland. In seagrass sediments, one unclassified *Agaricomycetes* genus from phylum *Basidiomycota* (3.3 % ± 3.1 %) and an unidentified *Saccharomycetales* genus (0.5 % ± 0.4 %) represented the common biomarker taxa.

Several functional guilds of prokaryotic microorganisms pertinent to wetland ecosystem processes were characterized and compared between different sites/ecosystems, including methanogens, anaerobic methane oxidizers, sulfur/sulfate-reducing microbes (SRM) and nitrifiers (Fig. 3).

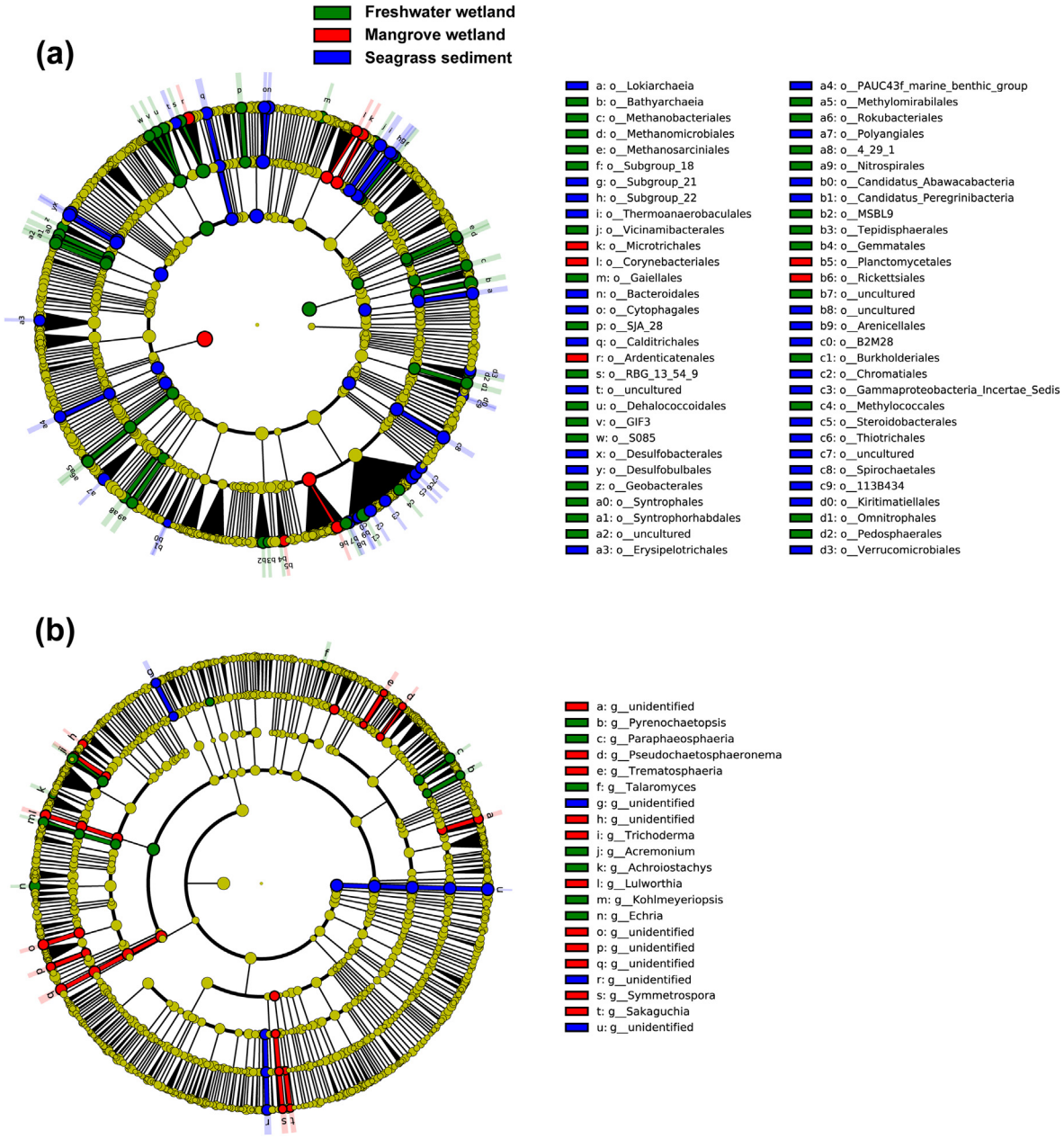


Fig. 2. Identification of prokaryotic (a) and fungal biomarker taxa (b) within each wetland types (freshwater wetland, mangrove forest and seagrass sediment) by LEfSe analysis of all bulk soil samples from February 2019 to October 2020. Cladograms indicate taxonomic classification of prokaryotic and fungal communities at order-level and genus-level, respectively. Taxa significantly more abundant in either freshwater marsh soil, mangrove soil, or seagrass bed are indicated in green, red and blue, respectively. Listing on the right of the cladogram corresponds to the letter matching taxa in the cladogram.

In each slough (SRS and TS/Ph), the relative abundance of methanogens decreased from freshwater wetlands transitioning to mangrove and/or seagrass habitats ($p < 0.001$, Fig. 3a). Correspondingly, there was also a compositional shift in major methanogenic phylotypes across different ecosystems. Particularly, genus *Methanobacterium* (within order *Methanobacteriales*), *Candidate* genus "Methanomethylicus" (*Methanobacteriales* order) and three genera from order of *Methanomicrobiales* comprised the majority of methanogens in freshwater, while an unclassified genus from order *Methanofastidiosales* exclusively dominated the methanogenic assemblage in seagrass sediments (Fig. 4). Putative denitrifying methanotrophs (*Methylomirabilales* order in NC10 phylum) showed ecosystem-specific relative abundance patterns similar to methanogens ($p = 0.003$) (Fig. 3b). In contrast, the SRM guild was present in higher proportions in seagrass sediments than in freshwater wetland soils ($p < 0.001$) (Fig. 3c). Proportions of aerobic nitrifiers, predominantly ammonia-oxidizing archaea, showed varying abundances between different sites ($p < 0.001$), but without ecosystem-specific pattern (Fig. 3d). Notably, in SRS nitrifiers were most frequently detected in mangrove wetland sites, whereas in TS/Ph their relative abundances were highest in freshwater wetland sites.

Higher richness (number of ASVs) was observed in SRS than in TS/Ph for prokaryotic communities ($p < 0.001$), but not for fungal communities ($p = 0.149$). No significant difference in microbial richness was observed between different sites/ecosystems within the same slough (Fig. S3).

3.2. Effect of seasonality on prokaryotic and fungal communities

All sampling sites were characterized by distinctive wet (June–November) and dry (December–May) seasons of South Florida, but PERMANOVA analysis

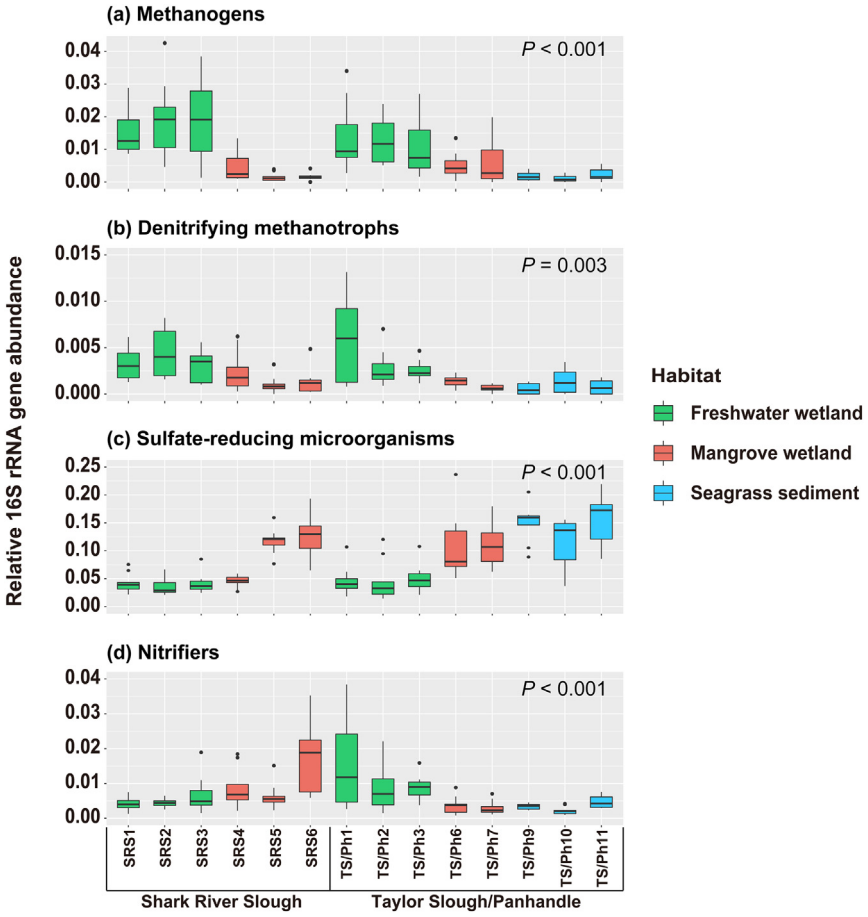


Fig. 3. Box plot showing relative abundances of functional prokaryotic guilds, including (a) methanogens, (b) denitrifying methanotrophs, (c) sulfate reducing microorganisms, and (d) nitrifiers, in 14 different wetland sites from two estuarine transects (Shark River Slough and Taylor Slough/Panhandle) from February 2019 to October 2020, representing three distinct wetland ecosystem types (freshwater wetland, mangrove forest and seagrass meadow). The upper and lower bounds of boxes correspond to the 25th and 75th percentiles, with a median line shown. Whiskers denote the 1.5 IQR (interquartile range) and dots represent outliers.

showed that such climate variations were not a significant influence on the microbial composition in the tested soils and sediments ($p = 0.057$ for prokaryotes and $p = 0.067$ for fungi). However, varying degrees of site-specific temporal shift in microbial composition were observed by calculating sample to centroid spread of weighted UniFrac distances of different sampling time points (Fig. 5). Temporal variations in prokaryotic community compositions were larger in mangrove wetland sites from TS/Ph compared to other sites ($p < 0.001$). Additionally, mangrove wetland and seagrass bed sediments from TS/Ph had the highest temporal variations in fungal community composition. In comparison, both prokaryotic and fugal community composition in freshwater site soils were less affected by seasonality.

The temporal variations of the major microbial taxa (average relative abundance >1 % for prokaryote and 0.5 % for fungi across all samples) in different sites were estimated by calculation of coefficient of variation in relative abundances. Among the 33 major prokaryotic taxa at order level, four orders, i.e., *Pseudomonadales* (within phylum *Proteobacteria*), *Bacillales* (phylum *Firmicutes*), *Flavobacteriales* (phylum *Bacteroidota*), and the Marine Benthic Group D (archaeal phylum *Thermoplasmatota*), showed the highest temporal variations (coefficient of variation of 103 %–188 %) among different sites (Fig. S4a). Among all 18 major fungal genera, *Podospora*, *Lulwoana*, *Lulworthia*, *Alternaria* (all within phylum of *Ascomycota*), *Gymnopilus* and *Vishniacozyma* (both within phylum *Basidiomycota*), had the highest temporal variations (coefficient of variation 201 %–271 %, Fig. S4b). Notably, *Gymnopilus* is a mushroom-forming fungus and its periodic booming in the seagrass sediments (Fig. S2) might be caused by spores, rather than in situ growth at the time of sampling.

3.3. Effect of soil depth on prokaryotic communities

Soil and sediment cores from August 2020 were analyzed at high resolution for depth-dependent vertical gradients in microbial communities using 16S rRNA gene sequences (Fig. S5). Consistent with results above, prokaryotic community structure strongly differed by ecosystem types (PERMANOVA, $p < 0.001$, Fig. S6), but surprisingly the prokaryotic community composition did not vary significantly along the soil depth (PERMANOVA, $p = 0.263$). Only a direct pairwise PERMANOVA comparison of the top (0–9 mm) and the bottom (72–81 mm) sections of the soil cores across different sites showed significant difference in microbial community composition ($p = 0.009$).

Analysis of LDA Effect Size (LEfSe) to characterize the microbial biomarkers in the top versus bottom sections of the soil cores revealed a total of 39 taxa (2, 8, 7, 9, 7, 6 taxa at domain, phylum, class, order, family and genus levels, respectively, LDA score ($\log_{10} > 3$) that were differentially distributed between top and bottom sections of the cores (Table S7). Specifically, total Archaea were more frequent in the bottom-most sections (12.6 % ± 1.2 %) than in the top-most sections (6.8 % ± 0.8 %), largely attributed to putative class *Bathyarchaeia* (8.2 % ± 0.9 % in bottom sections versus 4.3 % ± 0.7 % in top sections). *Bathyarchaeia* are poorly studied but have been suggested to harbor metabolic generalists adapted to anoxic environments (Zhou et al., 2018). Similarly, all other biomarker taxa with higher relative abundance in bottom sediments, including an unclassified *Syntrophobacterales* genus, an unclassified *Anaerolineae* genus, an uncultured *Dehalococcoidia* order, are known for an anaerobic lifestyle. In comparison, top sections of the cores had higher abundance

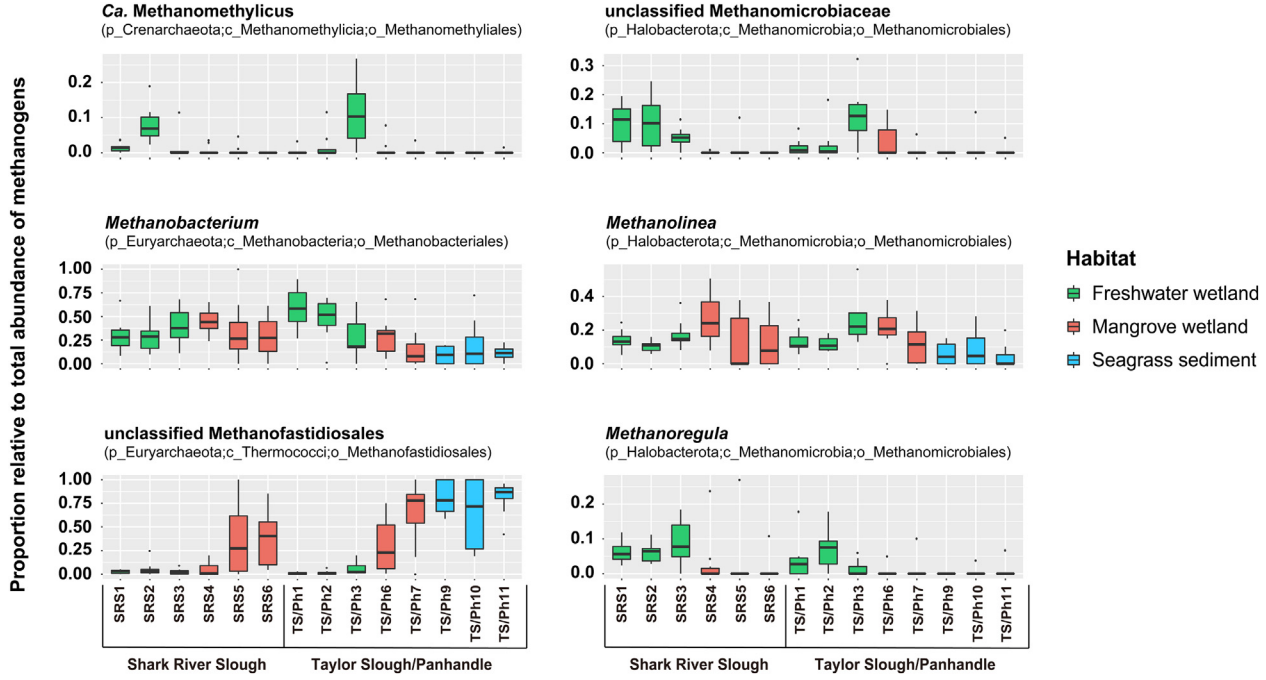


Fig. 4. Box plot displaying proportions of six major methanogenic genera relative to the total abundance of methanogens in 14 wetland sites based on taxonomic classification of 16S rRNA genes. The phylum, class and order affiliations of each genus are given in parentheses. Wetland sites are from two estuarine transects (Shark River Slough and Taylor Slough/Panhandle) representing three distinct wetland ecosystem types (freshwater wetland, mangrove forest and seagrass meadow). The upper and lower bounds of boxes correspond to the 25th and 75th percentiles, with a median line shown. Whiskers denote the 1.5 IQR (interquartile range) and dots represent outliers.

of photosynthetic *Cyanobacteria* and *Rhodobacteraceae* that contain facultatively anaerobic photoheterotrophs. Given that a significant proportion of detected microbial taxa in the cores were affiliated with primarily or strictly anaerobic microbial guilds, these results collectively suggest that the influence of oxygen and aerobic metabolism was limited to the top one cm of the soils and sediments.

The alpha diversity, including richness (observed ASVs) and evenness (Pielou's evenness), was significantly higher in top layer (section of 0–9 mm) than that in the deeper layers (sections between 36 and 81 mm) of the sediments ($p < 0.05$, Fig. S7). However, no change in alpha diversity was observed between different layers between 18- and 81-mm depths.

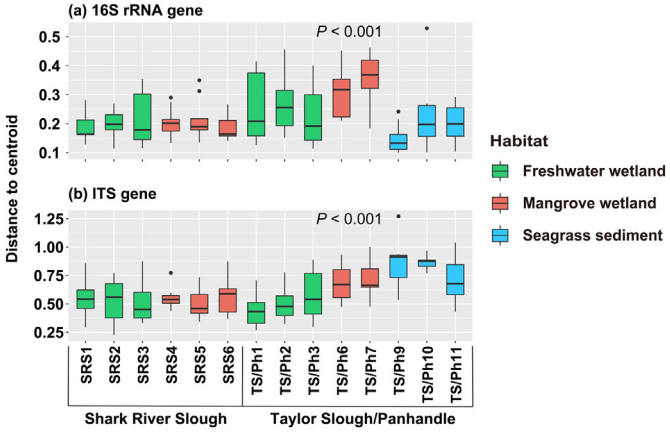


Fig. 5. Box plot showing temporal variations in beta-diversity of prokaryotic (a) and fungal (b) communities in each of the 14 wetland sites along Shark River Slough and Taylor Slough/Panhandle, as calculated by weighted UniFrac distances of samples to their site centroid based on 16S rRNA and ITS genes, respectively. The upper and lower bounds of boxes correspond to the 25th and 75th percentiles, with a median line shown. Whiskers denote the 1.5 IQR (interquartile range) and dots represent outliers.

3.4. Effect of simulated seawater intrusion on prokaryotic communities

Saltwater intrusions were simulated experimentally at two wetland sites along the southeastern boundary of SRS: a freshwater marsh site with no history of saltwater intrusion, and a brackish water marsh site with previous episodes of saltwater intrusion. Changes in prokaryotic communities following saltwater addition were monitored by sequencing of 16S rRNA genes. The saltwater addition did not change the richness ($p = 0.126$ for the freshwater site and $p = 0.584$ for the brackish site, two-way ANOVA followed by *post-hoc* Tukey test; pairwise *t*-test) of soil prokaryotic communities in both sites (Fig. S8). The overall prokaryotic community composition shifted in freshwater sediment soils ($p = 0.021$, PERMANOVA) but not in brackish marsh soils ($p = 0.127$) after exposure to saltwater (Fig. 6a and Fig. S9).

A total of 19 and 26 order-level biomarker taxa showed significant increase in abundance in salt-dosed treatments at the freshwater (Table S8) and brackish water wetland sites (Table S9), respectively, following saltwater addition. At the freshwater site, the biomarker taxa Marine Benthic Group D (1.54 % ± 0.23 %), *Aminicenantales* (1.10 % ± 0.21 %), and an unclassified *Thermodesulfovibrionia* order (1.13 % ± 0.35 %) became common taxa (relative abundance >1.0 %) after saltwater addition. Interestingly, no common taxa were identified as biomarker taxa in the brackish water marsh site, indicating the salt addition only affected rare taxa with relatively low abundance. Notably, two biomarker taxa in saltwater-dosed sites, *Calditrichales* and *Desulfobulbales*, were also found as the biomarker taxa in seagrass sediment of Florida Bay, demonstrating that the competitiveness of these taxa directly depends on salt concentration or associated sulfate supply. Among the functional guilds described above only SRM showed significant increase in relative abundance in the saltwater-dosed treatments ($p = 0.030$ and $p = 0.038$ in freshwater and brackish water

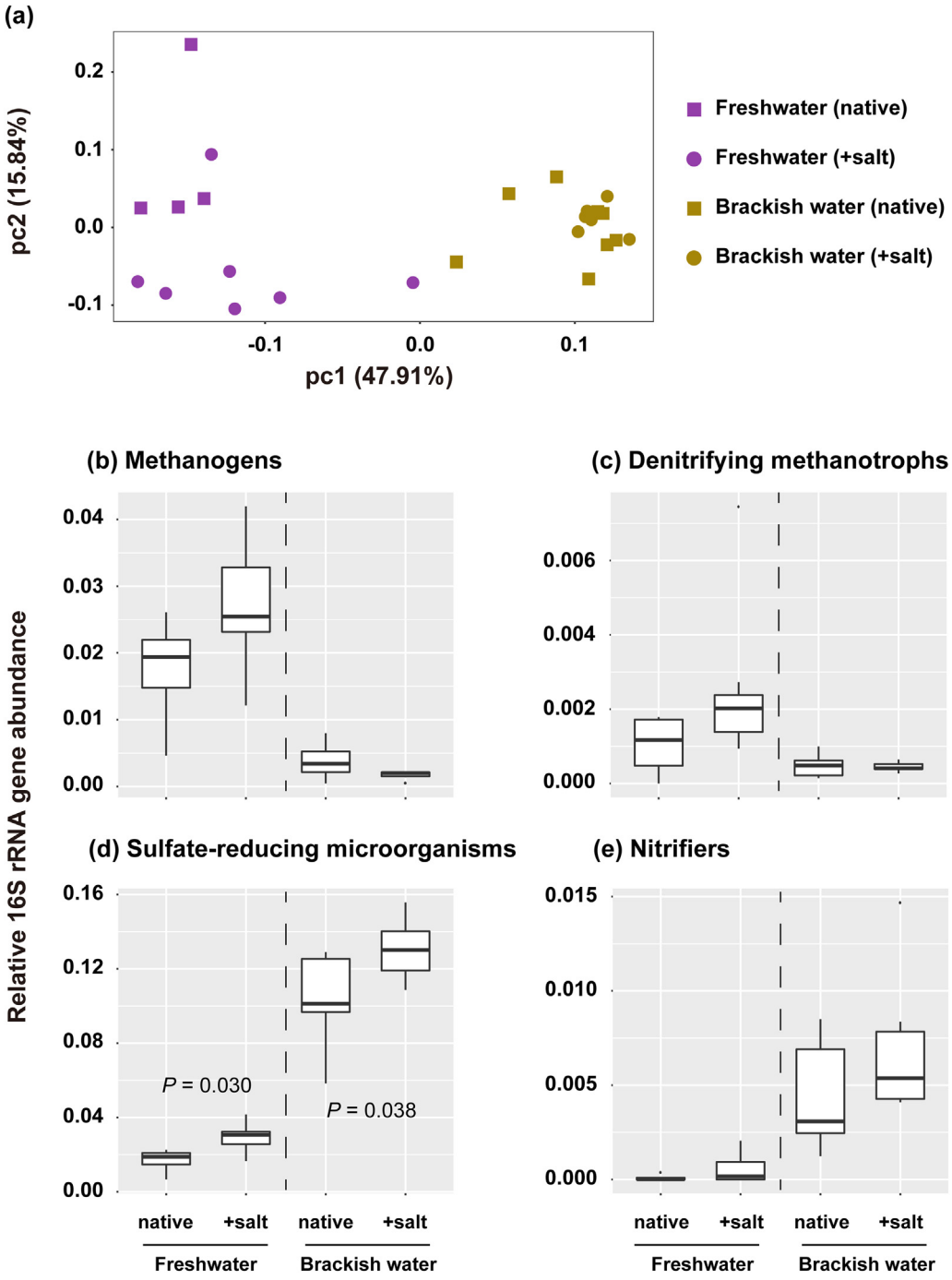


Fig. 6. Effect of pulsing salt intrusions on wetland soil microbial communities. Shown are PCoA of weighted UniFrac distances of total microbial communities based on 16S rRNA genes (a), box plots of the relative abundances of functional prokaryotic guilds, including (b) methanogens, (c) denitrifying methanotrophs, (d) sulfate reducing microorganisms, and (e) nitrifiers, in soils from a freshwater wetland and a brackish water wetland site in native ambient environments and after experiencing 4-year pulsing salt intrusions. The upper and lower bounds of boxes correspond to the 25th and 75th percentiles, with a median line shown. Whiskers denote the 1.5 IQR (interquartile range) and dots represent outliers. The *p* values are shown only when saltwater intrusion significantly changed the proportion of a genus at a site.

site, respectively), while relative abundances of the other three guilds remained stable (Fig. 6b–e). Although the relative abundance of total methanogens did not change, the composition shifted following saltwater treatment, as the proportion of the major methanogenic genus *Methanolinea* relative to all methanogens significantly decreased in both sites after saltwater addition (Fig. 7), suggesting either salt sensitivity or direct competition with SRM for carbon- and energy source.

4. Discussion

4.1. Factors shaping soil microbial community structure associated with different wetland types

The present study comprehensively assessed spatial and temporal variations in microbial diversity and composition in soils and sediments from $n = 14$ coastal wetland sites along the two main sloughs within the FCE,

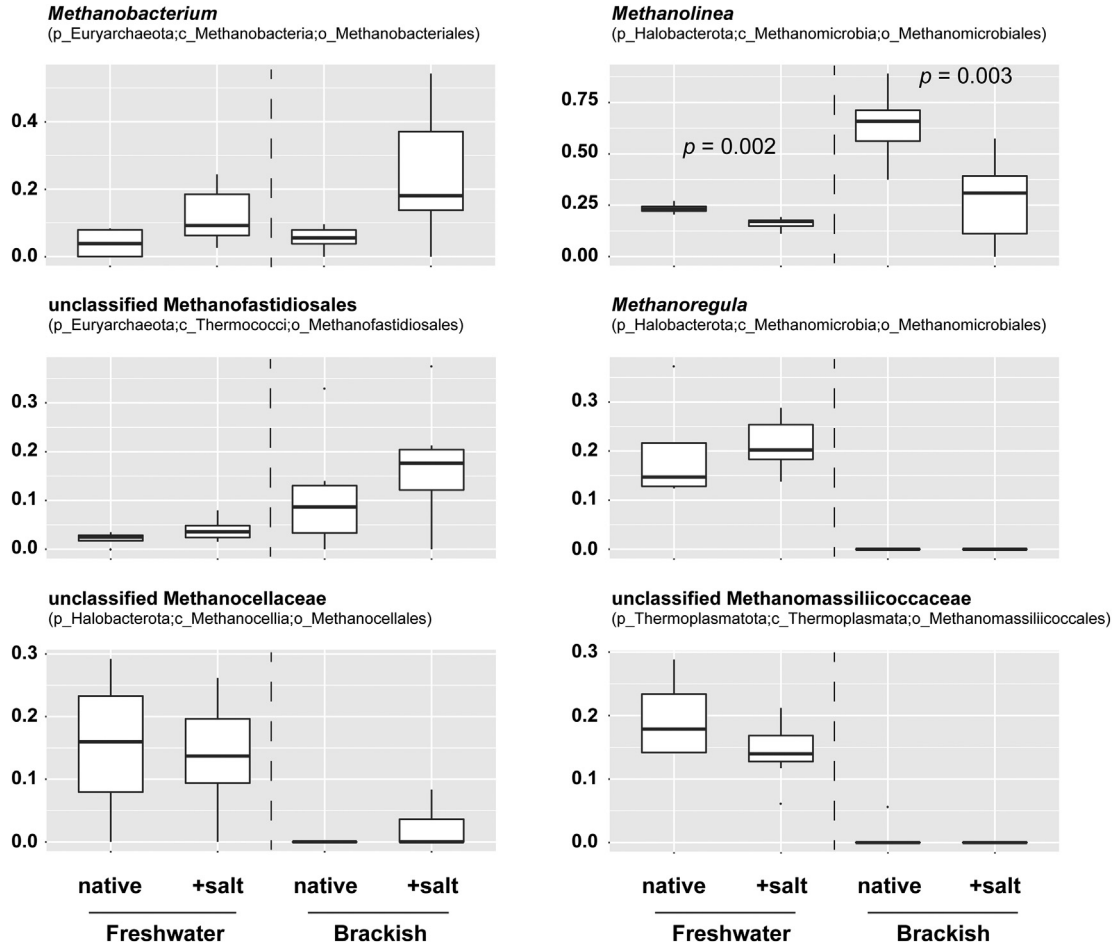


Fig. 7. Effect of experimental saltwater intrusion on the proportions of the six predominant methanogenic genera relative to the total relative abundance of methanogens. Shown are box plot of proportions of the six predominant methanogenic genera relative to the total relative abundance of methanogens in soils from a freshwater wetland and a brackish water wetland site with (+salt) or without (native) artificial saltwater treatment. The affiliations of phylum, class and order of each genus are given in parentheses. The upper and lower bounds of boxes correspond to the 25th and 75th percentiles, with a median line shown. Whiskers denote the 1.5 IQR (interquartile range) and dots represent outliers. A *p* value is shown where saltwater addition significantly changed the relative proportion of a genus.

spanning three large and distinct coastal wetland ecosystem types with apparent differences in hydrology, chemistry (e.g., salinity) and biology (e.g., plant community). Our results revealed that soil and sediment microbial community composition were vastly dominated by strictly anaerobic bacterial and archaeal taxa and notable presence of fungi in all sites and depths. Prokaryotic and fungal community composition was distinctively separated by ecosystem type, whereas seasonality and depth of sediment had much lesser influence on the microbial assemblage in these soils and sediments. These results collectively suggest that the rich microbial communities are not sensitive to short-term seasonal environmental fluctuations, e.g. freshwater inputs, or that short-term changes are not sufficiently resolved by the amplicon sequencing methodology employed here. However, microbial communities are restructured by saltwater intrusion leading to ecosystem and landscape changes.

The elevated salinity itself is most likely the main factor altering microbial community structure associated with conversion of freshwater wetlands in the future, for both prokaryotic and fungal communities. Salinities were 0.3 ± 0.0, 12.7 ± 1.6, 42.2 ± 2.1 psu in freshwater, mangrove and seagrass meadow wetlands, respectively (Table S1), which were associated with distinct prokaryotic and fungal community composition (Fig. 1). Salinity most likely had a direct impact on overall microbial communities in these wetland soils, since our four-year saltwater intrusion simulation has already led to significant change in microbial compositions (Figs. 6 and S9). This is further supported by similar salinity-depended changes of microbial community structure in the FCE water column (Laas et al., 2022), and independence of microbial community changes from specific plant cover in newly formed coastal wetlands (Zhang et al., 2021). Salinity also determined the microbial assemblage in desert ecosystem where plant growth was limited and had little effect on microbes (Zhang et al., 2019) and is therefore likely to directly influence soil microbial assemblages across different wetland ecosystems in this study. However, we cannot rule out potential direct effect of other environmental variables on microbial assembly, e.g., the different hydrology between a submerged seagrass meadows, intertidal mangrove and non-tidal freshwater marsh.

Additional variables besides salinity further structured microbial communities within freshwater and mangrove wetlands. SRS and TS/Ph sites are characterized by peat- and marl-based soils, respectively (Chen et al., 2013), with the freshwater soils from SRS having higher DOC and distinct DOC composition compared to TS/Ph soils (Chen et al., 2013; Regier et al., 2020). DOC concentrations potentially influenced both prokaryotic and fungal community composition in freshwater wetland sites leading to higher alpha diversity in SRS sites at higher DOC concentrations (Tables S3 and S4). However, similar DOC concentrations and microbial communities in mangrove forests from both sloughs, possibly due to interference of mangrove with DOC (Chen et al., 2013; Dittmar et al., 2006), suggest that organic matter richness and composition was a driver of community composition only in freshwater wetlands. Our results further revealed that phosphorus availability, either as soluble reactive P or total P, could have additionally structured the prokaryotic and fungal communities

within freshwater and mangrove wetlands. The FCE is known as oligotrophic phosphorous-limited wetland system (Noe and Childers, 2007) and all investigated sites showed TP (< 20 μg L^{-1}) and SRP (< 4 μg L^{-1}) concentrations typically considered as P-limited (Table S1). Interestingly, even within the low P concentration ranges microbial community composition in the freshwater and mangrove wetlands appeared to be sensitive to P variation (Tables S3 and S4). It may therefore be expected that further anthropogenic P loading to the Everglades or enhanced ecosystem restoration efforts could lead to significant changes in microbial composition and related ecological functions in Everglades wetlands (Bae et al., 2018; Chauhan et al., 2012; Wright et al., 2009), as well as in other upland and wetland habitats (Hartman et al., 2008; Huang et al., 2016; Liu et al., 2012).

4.2. Ecosystem-specific taxa reflect shift in biogeochemical functions

Several biogeochemically important functional microbial guilds showed ecosystem-specific patterns that were consistent with the environmental conditions, and significant shifts in relative frequency and phylogenetic composition of these guilds between ecosystem type, strongly suggesting alteration of corresponding biogeochemical processes. As expected, methanogens and SRM coexisted, but displayed opposite proportions in freshwater and saltwater-dominated ecosystems, respectively (Fig. 3). This is likely due to the competition of both groups for common substrates, e.g. hydrogen or acetate (Schink, 1997). In the absence of sulfate in freshwater wetlands, SRM likely thrive syntrophically with methanogens by fermenting sugars and organic acids to H_2 and acetate, which methanogens can subsequently use for methanogenesis. However, in presence of sulfate, SRM can achieve significantly higher growth yields by sulfate reduction, and thus outcompete methanogens (Lovley and Klug, 1983; Sela-Adler et al., 2017), leading to a shift from primarily methanogenic to sulfidogenic organic matter degradation at the transition from freshwater marshes to saltwater-influenced mangroves. Although sulfate was not analyzed directly here, previous research in the FCE system showed significantly higher chromium-reducible sulfide in mangrove soils and seagrass beds than in freshwater marshes, reflecting the much larger sulfate source and higher sulfate reduction activity in the saltwater influenced ecosystems (Chambers and Pederson, 2006), consistent with the observed shifts in methanogens and SRM reported in the seasonal survey here (Fig. 3). The observation of similar shifts in the saltwater intrusion experiment indicates that even at low salinities saltwater intrusions into previously pristine freshwater marshes may substantially alter the anaerobic microbial food web and the competition between methanogenic and sulfidogenic microbial organic matter degradation through enhanced sulfate availability.

Notably, we observed niche specialization among phylogenetically and metabolically distinct methanogens in different ecosystems (Fig. 4). The largest portion of methanogens in freshwater sediments was associated with the genus *Methanobacterium*, primarily formate- or H_2-dependent methanogens, whereas putatively methylotrophic "Ca. Methanofastidiosales" represented the primary methanogens in all seagrass sediments. Mangrove wetlands as the transitional ecosystem were dominated by *Methanobacterium and/or* "Ca. Methanofastidiosales". This shift in methanogenic composition suggests that major methane production pathways were different. Indeed, known members of "Ca. Methanofastidiosales" are obligate H_2-dependent methylotrophic methanogens that generate methane via methylated thiol reduction (Nobu et al., 2016), while many *Methanobacterium* sp. are formate or H_2-dependent, CO_2-reducing methanogens (Ferry, 2011). Similarly, according to regional and global biogeographic analyses of methanogens, *Methanobacterium* and other H_2-dependent, CO_2-reducing methanogens prevailed in terrestrial and transitional estuaries, including other areas of Florida Everglades, but were rarely found in marine habitats (Chauhan et al., 2004; Wang et al., 2019; Wen et al., 2017). In contrast, methylotrophic methanogens are frequently detected in seagrass beds (Cai et al., 2022; Maltby et al., 2018; Sun et al., 2020; Zhuang et al., 2018) and usually do not share common substrate with SRM (Oremland and Polcin, 1982). Interestingly, "Ca. Methanofastidiosales" also require H_2 for activity, and thus still may be affected by competition with SRM, which might explain their much lower abundance than SRM in all seagrass and some mangrove soils (Fig. 3a and c).

In addition to the functional guilds described above, many ecologically relevant prokaryotic taxa were found to specialize in different FCE wetland ecosystems. Markedly, an unclassified consortium of *Bathyarchaeota* species was more frequently detected in freshwater wetland soils than in mangrove and seagrass soils (Fig. 2a and Table S5). *Bathyarchaeota* are globally distributed archaea in anoxic environments including freshwater and marine sediments. Although they are poorly studied, they are known to contain members with wide metabolic capabilities, including acetogenesis (He et al., 2016) and methane metabolism (Evans et al., 2015). In the soils investigated here, relative frequencies of *Bathyarchaeota* were similar to non-bathyarchaeotal methanogenic and denitrifying methanotrophic guilds (Fig. 3a), suggesting similar freshwater adaptation of bathyarchaeotal organisms or possible interactions with methanogens via provision of fermentation products in these wetland soils. Conversely, unclassified *Lokiarchaeales* species displayed increased proportions along freshwater-mangrove-seagrass transects (Fig. 2a and Table S5). The first and only cultivated *Lokiarchaeales* strain can produce hydrogen and form syntrophic relationship with hydrogen-consuming SRM or methanogens (Imachi et al., 2020). It is thus reasonable to speculate that the *Lokiarchaeales* species could play an important role in supporting SRM and methanogens (e.g., obligate H_2-dependent "Ca. Methanofastidiosales") in these wetlands, since their relative proportions followed the similar ecosystem-related pattern (Figs. 3c and 4).

Fungal growth, metabolism and biogeochemical role in anaerobic wetland sediment soil remain poorly understood. Our study confirms dominance of *Ascomycota* (51.0 % ± 2.5 %) and *Basidiomycota* (13.6 % ± 1.8 %) found in other coastal habitats (Arfi et al., 2012; Grossart et al., 2019; Krauss et al., 2011; Picard, 2017; Xiao et al., 2020). Some fungal taxa showed clear ecosystem preference (Fig. 2b), e.g., much higher proportion of genus *Echria* were detected in freshwater soils (12.0 % ± 3.1 %) compared to mangrove (0.8 % ± 0.1 %) and seagrass (0.3 % ± 0.1 %) soils (Table S6). These findings corroborate the environmental sensitivity of *Echria* species, as they have previously been found exclusively in lentic, but not lotic Florida freshwater habitats (Raja et al., 2008; Raja and Shearer, 2006), which could make them a candidate microbial indicator to reflect the severity of freshwater wetland disruption in FCE. Several other fungal taxa, e.g. plant-pathogenic fungi may have originated from spores or submerged infected plant material, and may not reflect growth *in situ*.

4.3. Sensitivity of microbial communities to saltwater intrusion and environmental fluctuations

Saltwater intrusion is a major threat to coastal freshwater wetlands globally. The Everglades has historically been particularly vulnerable to saltwater intrusion due to declines in seasonal freshwater delivery (McIvor et al., 1994; Odum et al., 1995; Sklar and van der Valk, 2002), a naturally low topographic incline (Ross et al., 2000), and increasing rates of sea-level rise (Dessu et al., 2018; Wdowinski et al., 2016). Furthermore, an accelerating soil elevation loss has been observed in FCE, which is thought to be linked to microbial activities and loss in fine root biomass in association with saltwater intrusions (Chambers et al., 2014; Charles et al., 2019; Wilson et al., 2018). However, the responses of microbial communities to short-term salinity change and the connection to elevation loss are still not very well understood. To test the microbial response to saltwater intrusion, manipulations using saltwater addition were conducted in situ at a freshwater and a brackish wetland site in proximity to SRS. The results showed that microbial composition in freshwater wetland was sensitive to saltwater intrusion, whereas microbes in the brackish wetland with previous history of seawater intrusion did not change after exposure (Fig. 6a). The saltwater intrusion increased the relative abundance of SRM but did not alter methanogen abundance (Fig. 6b and d), thus potentially increasing sulfate reduction without instantly inhibiting methanogenesis, and thereby accelerating organic carbon mineralization

rates, as has been suggested in other freshwater wetlands experiencing saltwater intrusion (Chambers et al., 2011; Weston et al., 2010; Wilson et al., 2018). However, soil microbial extracellular enzyme activities at both freshwater an brackish water experimental saltwater dosing sites indicated that although pulses of saltwater had little effect on the enzyme activities and breakdown of soil organic matter (Servais et al., 2020), continued exposure to elevated salinity decreased beta-glucosidase and cellobiosidase activities, respiration rates, microbial biomass, and soil carbon content, leading to net C losses from coastal wetland soils (Servais et al., 2019). These changes could lead to loss of soil elevation in affected costal freshwater wetlands in FCE characterized with organic-rich soils and contribute to wetland "peat collapse" (Chambers et al., 2019). Intriguingly, certain SRM taxa were very responsive to elevated salinity, particularly the *Desulfobulbales*, regardless of the native ambient wetland condition and history (e.g., freshwater or brackish) (Fig. 6d). Although the relative abundance of total methanogens remained stable, the total biomass of methanogen could have decreased following the saltwater intrusion (He et al., 2022). Nevertheless, the compositional shift of soil methanogens in both sites after saltwater intrusion, especially, the declining of *Methanolinea* sp. suggests low competitiveness with SRM (Fig. 7). These functional guilds or particular taxa thus might be useful sensitive bioindicators for disturbance of biogeochemical pathways at the early stage of sea level rise-related salt water intrusion, even if significant changes in the net ecosystem services (e.g., net methanogenesis or mineralization rate) might not have been observed yet (Neubauer, 2013).

Wetland sediments are characterized by temporal heterogeneity, which also led to variations in the structure of soil microbial communities, as revealed by year-around monitoring of both prokaryotic and fungal communities in the present study. South Florida is characterized by tropical climate, with distinct wet (June–November) and dry (December–May) seasons. Many critical environmental parameters, including temperature, water level and salinity (impacted by precipitation and tidal activity), fluctuated with changing seasons, potentially altering microbial activity and leading to variation in microbial composition. However, the relatively minor response of microbial composition to seasonality (Table S2) suggested certain resistance of wetland soil communities to moderate environmental fluctuations, or lack of detection by the employed amplicon sequencing approach (see above). Similarly, relatively little temporal heterogeneity was observed in microbial communities in agricultural soils (Orellana et al., 2018) and other coastal wetland sediments (Behera et al., 2019), but seemed to cause more apparent microbial compositional variation in lake water samples (Crump et al., 2009; Shabarova et al., 2021; Shade et al., 2007). This might be explained by (i) greater metabolic versatility of soil microbes in response to changing environments (Konstantinidis and Tiedje, 2004; Orellana et al., 2018); (ii) higher frequency of dormancy of soil microbes compared to aquatic microbial communities (Lennon and Jones, 2011); (iii) greater mobility of microbes in lake systems than in soils (Shabarova et al., 2021); and (iv) large proportions of extracellular or relic DNA present in soils, obscuring the estimation of microbial diversity, especially hindering the detection of temporal variability of microbial communities (Carini et al., 2020; Carini et al., 2016).

Soil depth was reported to have a significant impact on prokaryotic microbial distribution. For instance, bacterial compositional dissimilarity between the surface and subsurface soils was equivalent to horizontal differences over several kilometers in plateau soils (Chu et al., 2016), while distinctive depth-specific patterns of prokaryotic structure and activity were frequently observed in wetland sediments with association to varied light and nutrient availability (Boer et al., 2009; Li et al., 2022; Qiao et al., 2018; Shen et al., 2015). However, these observations of depth-related shift in prokaryotic community structure were all based on relatively large vertical distances (at least 10-cm difference between sectioned soil core samples), while the sensitivity of microbial assemblage to small-scale vertical distance change remained unclear. Our results show that depth has much less influence on microbial composition in the top 10-cm sediment core, compared to impact of horizontal distance (freshwater-mangrove-seagrass transects, Fig. S6). Nevertheless, a number of light-dependent biomarker taxa were found in top (~0–0.9 cm) layers of the sampled soil cores and were absent in deeper layers. Although we could not monitor the oxygen distribution across soil depth, previous studies showed oxygen diffusion decreased with depth and all oxygen was consumed within 2–5-cm depth from the sediment-water interface in vegetated coastal wetland soils and shelf sea sediments (Brodersen et al., 2019; Silburn et al., 2017). Our results suggest that the influence of oxygen in the investigated wetland soils within the Florida Everglades were limited to the uppermost cm, and that the soil beneath remained largely anoxic throughout the year.

5. Conclusions

Coastal wetlands are under pressure of changes due to sea level rise. Our results provide the first detailed soil prokaryotic and fungal community analyses across the main Florida Everglades transects. Soil microbial communities within the Everglades were distinct between ecosystem types, and aside from salinity are structured by soil phosphorous and organic matter quality and quantity. Soil microbial communities appear relatively well adapted to temporal or moderate environmental fluctuations (e.g. temperature, moisture) and a certain degree of global change-induced environmental disturbances (e.g., heatwaves, flooding and storms). In contrast, chemical alterations (increased salinity, increased sulfate availability) due to saltwater intrusions result in detectable microbial community shifts in particular of methanogenic and sulfate-reducing microbial assemblages even at low overall salinities. These biomarker taxa may help detect saltwater intrusion effects even before significant ecosystem changes or loss of soil elevation have been observed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159865.

CRediT authorship contribution statement

Jun Zhao: Data curation, Formal analysis, Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Seemanti Chakrabarti:** Data curation, Formal analysis, Investigation. **Randolph Chambers:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Pamela Weisenhorn:** Formal analysis, Investigation, Writing – review & editing. **Rafael Travieso:** Data curation, Project administration, Investigation. **Sandro Stumpf:** Data curation, Project administration, Investigation. **Emily Standen:** Project administration, Investigation. **Henry Briceno:** Data curation, Project administration, Writing – review & editing. **Tiffany Troxler:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Evelyn Gaiser:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **John Kominoski:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Braham Dhillon:** Formal analysis, Writing – review & editing. **Willm Martens-Habbena:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Data availability

All data generated have been deposited in public databases or are attached as supplemental materials included in the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Science Foundation Florida Coastal Everglades LTER program (DEB-1237517, DEB-1832229), Florida

Sea Grant (R/C-S-86), South Florida Water Management District, and Everglades National Park. Further support came from Florida Agricultural Experiment Station (Hatch project FLA-FTL-005680), and an UF IFAS Early Career Award to WMH. This is contribution #1496 of the Institute of the Environment at Florida International University.

References

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245–253.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 129–137.

Arfi, Y., Marchand, C., Wartel, M., Record, E., 2012. Fungal diversity in anoxic-sulfidic sediments in a mangrove soil. Fungal Ecol. 5, 282–285.

Bae, H.S., Morrison, E., Chanton, J.P., Ogram, A., 2018. Methanogens are major contributors to nitrogen fixation in soils of the Florida Everglades. Appl. Environ. Microbiol. 84, e02222-17.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81, 169–193.

Behera, P., Mohapatra, M., Kim, J.Y., Adhya, T.K., Pattnaik, A.K., Rastogi, G., 2019. Spatial and temporal heterogeneity in the structure and function of sediment bacterial communities of a tropical mangrove forest. Environ. Sci. Pollut. Res. 26, 3893–3908.

Boer, S.I., Hedtkamp, S.I., van Beusekom, J.E., Fuhrman, J.A., Boetius, A., Ramette, A., 2009. Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sands. ISME J. 3, 780–791.

Brodersen, K.E., Trevathan-Tackett, S.M., Nielsen, D.A., Connolly, R.M., Lovelock, C.E., Atwood, T.B., et al., 2019. Oxygen consumption and sulfate reduction in vegetated coastal habitats: effects of physical disturbance. Front. Mar. Sci. 6, 14.

Cai, M., Yin, X., Tang, X., Zhang, C., Zheng, Q., Li, M., 2022. Metatranscriptomics reveals different features of methanogenic archaea among global vegetated coastal ecosystems. Sci. Total Environ. 802, 149848.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Cao, L., Zhou, Z., Xu, X., Shi, F., 2020. Spatial and temporal variations of the greenhouse gas emissions in coastal saline wetlands in southeastern China. Environ. Sci. Pollut. Res. 27, 1118–1130.

Caporaso, J.G., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat. Microbiol. 2, 16242.

Carini, P., Delgado-Baquerizo, M., Hinckley Eve-Lyn, S., Holland-Moritz, H., Brewer Tess, E., Rue, G., et al., 2020. Effects of spatial variability and relic DNA removal on the detection of temporal dynamics in soil microbial communities. mBio 11, e02776-19.

Chambers, R.M., Pederson, K.A., 2006. Variation in soil phosphorus, sulfur, and iron pools among south Florida wetlands. Hydrobiologia 569, 63–70.

Chambers, L.G., Reddy, K.R., Osborne, T.Z., 2011. Short-term response of carbon cycling to salinity pulses in a freshwater wetland. Soil Sci. Soc. Am. J. 75, 2000–2007.

Chambers, L.G., Davis, S.E., Troxler, T.G., 2014. Sea level rise in the Everglades: plant-soil-microbial feedbacks in response to changing physical conditions. In: Entry, J.A., Gottlieb, A.D., Jayarchandran, K., Ogram, A. (Eds.), Microbiology of the Everglades Ecosystem, 1st edition CRC Press, Boca Raton.

Chambers, L.G., Steinmuller, H.E., Breithaupt, J.L., 2019. Toward a mechanistic understanding of "peat collapse" and its potential contribution to coastal wetland loss. Ecology 100, e02720.

Charles, S.P., Kominoski, J.S., Troxler, T.G., Gaiser, E.E., Servais, S., Wilson, B.J., et al., 2019. Experimental saltwater intrusion drives rapid soil elevation and carbon loss in freshwater and brackish everglades marshes. Estuar. Coasts 42, 1868–1881.

Chauhan, A., Ogram, A., Reddy, K.R., 2004. Syntrophic-methanogenic associations along a nutrient gradient in the Florida Everglades. Appl. Environ. Microbiol. 70, 3475–3484.

Chauhan, A., Pathak, A., Ogram, A., 2012. Composition of methane-oxidizing bacterial communities as a function of nutrient loading in the Florida everglades. Microb. Ecol. 64, 750–759.

Chen, M., Maie, N., Parish, K., Jaffé, R., 2013. Spatial and temporal variability of dissolved organic matter quantity and composition in an oligotrophic subtropical coastal wetland. Biogeochemistry 115, 167–183.

Chen, Q., Guo, B., Zhao, C., Xing, B., 2018. Characteristics of CH_4 and CO_2 emissions and influence of water and salinity in the Yellow River delta wetland, China. Environ. Pollut. 239, 289–299.

Cheung, M.K., Wong, C.K., Chu, K.H., Kwan, H.S., 2018. Community structure, dynamics and interactions of bacteria, archaea and fungi in subtropical coastal wetland sediments. Sci. Rep. 8, 14397.

Chi, Z., Wang, W., Li, H., Wu, H., Yan, B., 2021a. Soil organic matter and salinity as critical factors affecting the bacterial community and function of Phragmites australis dominated riparian and coastal wetlands. Sci. Total Environ. 762, 143156.

Chi, Z., Zhu, Y., Li, H., Wu, H., Yan, B., 2021b. Unraveling bacterial community structure and function and their links with natural salinity gradient in the Yellow River Delta. Sci. Total Environ. 773, 145673.

Childers, D.L., Boyer, J.N., Davis, S.E., Madden, C.J., Rudnick, D.T., Sklar, F.H., 2006. Relating precipitation and water management to nutrient concentrations in the oligotrophic "up-side-down" estuaries of the Florida Everglades. Limnol. Oceanogr. 51, 602–616.

Chmura, G.L., Anisfeld, S.C., Cahoon, D.R., Lynch, J.C., 2003. Global carbon sequestration in tidal, saline wetland soils. Glob. Biogeochem. Cycles 17, 1111.

Chu, H., Sun, H., Tripathi, B.M., Adams, J.M., Huang, R., Zhang, Y., et al., 2016. Bacterial community dissimilarity between the surface and subsurface soils equals horizontal differences over several kilometers in the western Tibetan Plateau. Environ. Microbiol. 18, 1523–1533.

Church, J.A., White, N.J., 2011. Sea-level rise from the late 19th to the early 21st century. Surv. Geophys. 32, 585–602.

Craft, C., Clough, J., Ehman, J., Joye, S., Park, R., Pennings, S., et al., 2009. Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. Front. Ecol. Environ. 7, 73–78.

Crump, B.C., Peterson, B.J., Raymond, P.A., Amon, R.M.W., Rinehart, A., McClelland, J.W., et al., 2009. Circumpolar synchrony in big river bacterioplankton. Proc. Natl. Acad. Sci. U. S. A. 106, 21208–21212.

Dessu, S.B., Price, R.M., Troxler, T.G., Kominoski, J.S., 2018. Effects of sea-level rise and freshwater management on long-term water levels and water quality in the Florida Coastal Everglades. J. Environ. Manag. 211, 164–176.

Dittmar, T., Hertkorn, N., Kattner, G., Lara, R.J., 2006. Mangroves, a major source of dissolved organic carbon to the oceans. Glob. Biogeochem. Cycles 20, GB1012.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930.

Evans, P.N., Parks, D.H., Chadwick, G.L., Robbins, S.J., Orphan, V.J., Golding, S.D., et al., 2015. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. Science 350, 434–438.

Ferry, J.G., 2011. Fundamentals of methanogenic pathways that are key to the biomethanation of complex biomass. Curr. Opin. Biotechnol. 22, 351–357.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Grossart, H.P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M., Rojas-Jimenez, K., 2019. Fungi in aquatic ecosystems. Nat. Rev. Microbiol. 17, 339–354.

Han, G., Sun, B., Chu, X., Xing, Q., Song, W., Xia, J., 2018. Precipitation events reduce soil respiration in a coastal wetland based on four-year continuous field measurements. Agric. For. Meteorol. 256–257, 292–303.

Hartman, W.H., Richardson, C.J., Vilgalys, R., Bruland, G.L., 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proc. Natl. Acad. Sci. U. S. A. 105, 17842–17847.

He, Y., Li, M., Perumal, V., Feng, X., Fang, J., Xie, J., et al., 2016. Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. Nat. Microbiol. 1, 16035.

He, L., She, C., Huang, J., Yang, P., Yu, H., Tong, C., 2022. Effects of constant and fluctuating saltwater addition on CH_4 fluxes and methanogens of a tidal freshwater wetland: a mesocosm study. Estuar. Coast. Shelf Sci. 277, 108076.

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, 206.

Hopkinson, C.S., Wolanski, E., Cahoon, D.R., Perillo, G.M.E., Brinson, M.M., 2019. Chapter 1 - Coastal wetlands: A synthesis. In: Perillo, G.M.E., Wolanski, E., Cahoon, D.R., Hopkinson, C.S. (Eds.), Coastal Wetlands. Elsevier, pp. 1–75.

Huang, J., Hu, B., Qi, K., Chen, W., Pang, X., Bao, W., et al., 2016. Effects of phosphorus addition on soil microbial biomass and community composition in a subalpine spruce plantation. Eur. J. Soil Biol. 72, 35–41.

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. Microb. Ecol. 59, 284–295.

Imachi, H., Nobu, M.K., Nakahara, N., Morono, Y., Ogawara, M., Takaki, Y., et al., 2020. Isolation of an archaeon at the prokaryote-eukaryote interface. Nature 577, 519–525.

Konstantinidis, K.T., Tiedje, J.M., 2004. Trends between gene content and genome size in prokaryotic species with larger genomes. Proc. Natl. Acad. Sci. U. S. A. 101, 3160–3165.

Krauss, G.J., Sole, M., Krauss, G., Schlosser, D., Wesenberg, D., Barlocher, F., 2011. Fungi in freshwaters: ecology, physiology and biochemical potential. FEMS Microbiol. Rev. 35, 620–651.

Laas, P., Ugarelli, K., Travieso, R., Stumpf, S., Gaiser, E.E., Kominoski, J.S., et al., 2022. Water column microbial communities vary along salinity gradients in the Florida Coastal Everglades wetlands. Microorganisms 10, 215.

Larkin, D.J., 2018. Wetland heterogeneity. In: Finlayson, C.M., Everard, M., Irvine, K., McInnes, R.J., Middleton, B.A., van Dam, A.A., et al. (Eds.), The Wetland Book: I: Structure and Function, Management, and Methods. Springer, Dordrecht, Netherlands, pp. 177–182.

Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat. Rev. Microbiol. 9, 119–130.

Li, W., Lv, X., Ruan, J., Yu, M., Song, Y.B., Yu, J., et al., 2018. Variations in soil bacterial composition and diversity in newly formed coastal wetlands. Front. Microbiol. 9, 3256.

Li, H., Chi, Z., Li, J., Wu, H., Yan, B., 2019. Bacterial community structure and function in soils from tidal freshwater wetlands in a Chinese delta: potential impacts of salinity and nutrient. Sci. Total Environ. 696, 134029.

Li, H., Liang, S., Chi, Z., Wu, H., Yan, B., 2022. Unveiling microbial community and function involved in anammox in paddy vadose under groundwater irrigation. Sci. Total Environ. 849, 157876.

Liu, L., Gundersen, P., Zhang, T., Mo, J., 2012. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. Soil Biol. Biochem. 44, 31–38.

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 Biol. Biochem. 112, 56–67.

Logozzo, L., Tzortziou, M., Neale, P., Clark, J.B., 2021. Photochemical and microbial degradation of chromophoric dissolved organic matter exported from tidal marshes. J. Geophys. Res. Biogeosci. 126, e2020JG005744.

Lovley, D.R., Klug, M.J., 1983. Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. 45, 187–192.
Lu, Q., Bai, J., Gao, Z., Wang, J., Zhao, Q., 2015. Effects of water level and salinity on total sulfur contents in salt marsh soils of the Yellow River Delta, China. Wetlands 36, 137–143.
Maietta, C.E., Hondula, K.L., Jones, C.N., Palmer, M.A., 2020. Hydrological conditions influence soil and methane-cycling microbial populations in seasonally saturated wetlands. Front. Environ. Sci. 8, 593942.
Maltby, J., Steinle, L., Löscher, C.R., Bange, H.W., Fischer, M.A., Schmidt, M., et al., 2018. Microbial methanogenesis in the sulfate-reducing zone of sediments in the Eckernförde Bay, SW Baltic Sea. Biogeosciences 15, 137–157.
McIvor, C., Ley, J.A., Bjork, R., 1994. Changes in freshwater inflow from the Everglades to Florida Bay including effects on biota and biotic processes: a review. Everglades. The Ecosystem and Its Restoration. ST. Lucie Press, Boca Raton, FL, USA, pp. 117–146.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. Plos One 8, 11.
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. Glob. Chang. Biol. 20, 1351–1362.
Neubauer, S.C., 2013. Ecosystem responses of a tidal freshwater marsh experiencing saltwater intrusion and altered hydrology. Estuar. Coasts 36, 491–507.
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 J. 10, 2478–2487.
Noe, G.B., Childers, D.L., 2007. Phosphorus budgets in Everglades wetland ecosystems: the effects of hydrology and nutrient enrichment. Wetl. Ecol. Manag. 15, 189–205.
Odum, W.E., Odum, E.P., Odum, H.T., 1995. Nature's pulsing paradigm. Estuaries 18, 547.
Orellana, L.H., Chee-Sanford, J.C., Sanford, R.A., Loffler, F.E., Konstantinidis, K.T., 2018. Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. Appl. Environ. Microbiol. 84, e01646-01617.
Oremland, R.S., Polcin, S., 1982. Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. Appl. Environ. Microbiol. 44, 1270–1276.
Osland, M.J., Gabler, C.A., Grace, J.B., Day, R.H., McCoy, M.L., McLeod, J.L., et al., 2018. Climate and plant controls on soil organic matter in coastal wetlands. Glob. Chang. Biol. 24, 5361–5379.
Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414.
Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., et al., 2011. Scikit-learn: machine learning in Python. J. Mach. Learn. Res. 12, 2825–2830.
Picard, K.T., 2017. Coastal marine habitats harbor novel early-diverging fungal diversity. Fungal Ecol. 25, 1–13.
Poffenbarger, H.J., Needelman, B.A., Megonigal, J.P., 2011. Salinity influence on methane emissions from tidal marshes. Wetlands 31, 831–842.
Qiao, Y., Liu, J., Zhao, M., Zhang, X.H., 2018. Sediment depth-dependent spatial variations of bacterial communities in mud deposits of the eastern China marginal seas. Front. Microbiol. 9, 1128.
Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing noise from pyrosequenced amplicons. BMC Bioinformatics 12, 38.
R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.
Raja, H.A., Shearer, C.A., 2006. *Arnium gigantosporum*, a new ascomycete species from fresh water in Florida. Fungal Divers. 22, 219–225.
Raja, H.A., Schmit, J.P., Shearer, C.A., 2008. Latitudinal, habitat and substrate distribution patterns of freshwater ascomycetes in the Florida Peninsula. Biodivers. Conserv. 18, 419–455.
Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: a review. Soil Biol. Biochem. 81, 108–123.
Regier, P., Larsen, L., Cawley, K., Jaffé, R., 2020. Linking hydrology and dissolved organic matter characteristics in a subtropical wetland: a long-term study of the Florida Everglades. Glob. Biogeochem. Cycles 34, e2020GB006648.
Rosentreter, J.A., Al-Haj, A.N., Fulweiler, R.W., Williamson, P., 2021. Methane and nitrous oxide emissions complicate coastal blue carbon assessments. Glob. Biogeochem. Cycles 35, e2020GB006858.
Ross, M.S., Meeder, J.F., Sah, J.P., Ruiz, P.L., Telesnicki, G.J., 2000. The Southeast Saline Everglades revisited: 50 years of coastal vegetation change. J. Veg. Sci. 11, 101–112.
Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol. Mol. Biol. Rev. 61, 262–280.
Schoepfer, V.A., Bernhardt, E.S., Burgin, A.J., 2014. Iron clad wetlands: soil iron-sulfur buffering determines coastal wetland response to salt water incursion. J. Geophys. Res. Biogeosci. 119, 2209–2219.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., et al., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60.
Sela-Adler, M., Ronen, Z., Herut, B., Antler, G., Vigderovich, H., Eckert, W., et al., 2017. Co-existence of methanogenesis and sulfate reduction with common substrates in sulfate-rich estuarine sediments. Front. Microbiol. 8, 766.
Servais, S., Kominoski, J.S., Charles, S.P., Gaiser, E.E., Mazzei, V., Troxler, T.G., et al., 2019. Saltwater intrusion and soil carbon loss: testing effects of salinity and phosphorus loading on microbial functions in experimental freshwater wetlands. Geoderma 337, 1291–1300.
Servais, S., Kominoski, J.S., Coronado-Molina, C., Bauman, L., Davis, S.E., Gaiser, E.E., et al., 2020. Effects of saltwater pulses on soil microbial enzymes and organic matter breakdown in freshwater and brackish coastal wetlands. Estuar. Coasts 43, 814–830.
Shabarova, T., Salcher, M.M., Porcal, P., Znachor, P., Nedoma, J., Grossart, H.P., et al., 2021. Recovery of freshwater microbial communities after extreme rain events is mediated by cyclic succession. Nat. Microbiol. 6, 479–488.
Shade, A., Kent, A.D., Jones, S.E., Newton, R.J., Triplett, E.W., McMahon, K.D., 2007. Interannual dynamics and phenology of bacterial communities in a eutrophic lake. Limnol. Oceanogr. 52, 487–494.
Shelton, S., Neale, P., Pinsonneault, A., Tzortziou, M., 2021. Biodegradation and photodegradation of vegetation-derived dissolved organic matter in tidal marsh ecosystems. Estuar. Coasts 45, 1324–1342.
Shen, L.-d., Liu, S., He, Z.-f., Lian, X., Huang, Q., He, Y.-f., et al., 2015. Depth-specific distribution and importance of nitrite-dependent anaerobic ammonium and methane-oxidising bacteria in an urban wetland. Soil Biol. Biochem. 83, 43–51.
Silburn, B., Kroger, S., Parker, E.R., Sivyer, D.B., Hicks, N., Powell, C.F., et al., 2017. Benthic pH gradients across a range of shelf sea sediment types linked to sediment characteristics and seasonal variability. Biogeochemistry 135, 69–88.
Sklar, F.H., van der Valk, A., 2002. Tree islands of the Everglades: an overview. In: Sklar, F.H., van der Valk, A. (Eds.), Tree Islands of the Everglades. Springer, Dordrecht, Netherlands, pp. 1–18.
Solorzano, L., Sharp, J.H., 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25, 754–757.
Spivak, A.C., Sanderman, J., Bowen, J.L., Canuel, E.A., Hopkinson, C.S., 2019. Global-change controls on soil-carbon accumulation and loss in coastal vegetated ecosystems. Nat. Geosci. 12, 685–692.
Sun, Y., Song, Z., Zhang, H., Liu, P., Hu, X., 2020. Seagrass vegetation affect the vertical organization of microbial communities in sediment. Mar. Environ. Res. 162, 105174.
Tan, L., Ge, Z., Zhou, X., Li, S., Li, X., Tang, J., 2020. Conversion of coastal wetlands, riparian wetlands, and peatlands increases greenhouse gas emissions: a global meta-analysis. Glob. Chang. Biol. 26, 1638–1653.
Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., et al., 2017. A communal catalogue reveals Earth's multiscale microbial diversity. Nature 551, 457–463.
Wang, B., Liu, F., Zheng, S., Hao, Q., 2019. Trophic strategy of diverse methanogens across a river-to-sea gradient. J. Microbiol. 57, 470–478.
Wdowinski, S., Bray, R., Kirtman, B.P., Wu, Z., 2016. Increasing flooding hazard in coastal communities due to rising sea level: case study of Miami Beach, Florida. Ocean Coast. Manag. 126, 1–8.
Weissman, D.S., Tully, K.L., 2020. Saltwater intrusion affects nutrient concentrations in soil porewater and surface waters of coastal habitats. Ecosphere 11, e03041.
Welti, N., Hayes, M., Lockington, D., 2016. Seasonal nitrous oxide and methane emissions across a subtropical estuarine salinity gradient. Biogeochemistry 132, 55–69.
Wen, X., Yang, S., Horn, F., Winkel, M., Wagner, D., Liebner, S., 2017. Global biogeographic analysis of methanogenic archaea identifies community-shaping environmental factors of natural environments. Front. Microbiol. 8, 1339.
Weston, N.B., Vile, M.A., Neubauer, S.C., Velinsky, D.J., 2010. Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. Biogeochemistry 102, 135–151.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, London, pp. 315–322.
Wilson, B.J., Servais, S., Mazzei, V., Kominoski, J.S., Hu, M., Davis, S.E., et al., 2018. Salinity pulses interact with seasonal dry-down to increase ecosystem carbon loss in marshes of the Florida Everglades. Ecol. Appl. 28, 2092–2108.
Wilson, B.J., Servais, S., Charles, S.P., Mazzei, V., Gaiser, E.E., Kominoski, J.S., et al., 2019. Phosphorus alleviation of salinity stress: effects of saltwater intrusion on an Everglades freshwater peat marsh. Ecology 100, 15.
Wright, A.L., Ramesh Reddy, K., Newman, S., 2009. Microbial indicators of eutrophication in Everglades wetlands. Soil Sci. Soc. Am. J. 73, 1597–1603.
Xiao, R., Guo, Y., Zhang, M., Pan, W., Wang, J.J., 2020. Stronger network connectivity with lower diversity of soil fungal community was presented in coastal marshes after sixteen years of freshwater restoration. Sci. Total Environ. 744, 140623.
Zhang, K., Shi, Y., Cui, X., Yue, P., Li, K., Liu, X., et al., 2019. Salinity is a key determinant for soil microbial communities in a desert ecosystem. mSystems 4, e00225-00218.
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. Environ. Microbiol. 23, 1020–1037.
Zhou, M., Butterbach-Bahl, K., Vereecken, H., Bruggemann, N., 2017. A meta-analysis of soil salinization effects on nitrogen pools, cycles and fluxes in coastal ecosystems. Glob. Chang. Biol. 23, 1338–1352.
Zhou, Z., Pan, J., Wang, F., Gu, J.D., Li, M., 2018. Bathyarchaeota: globally distributed metabolic generalists in anoxic environments. FEMS Microbiol. Rev. 42, 639–655.
Zhuang, G.-C., Heuer, V.B., Lazar, C.S., Goldhammer, T., Wendt, J., Samarkin, V.A., et al., 2018. Relative importance of methylotrophic methanogenesis in sediments of the Western Mediterranean Sea. Geochim. Cosmochim. Acta 224, 171–186.