ORIGINAL RESEARCH

Effects of Salinity and Inundation on Microbial Community Structure and Function in a Mangrove Peat Soil

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Abstract Shifts in microbial community function and structure can be indicators of environmental stress and ecosystem change in wetland soils. This study evaluated the effects of increased salinity, increased inundation, and their combination, on soil microbial function (enzyme activity) and structure (phospholipid fatty acid (PLFA) signatures and terminal restriction fragment length polymorphisms (T-RFLP) profiles) in a brackish mangrove peat soil using tidal mesocosms (Everglades, Florida, USA). Increased tidal inundation resulted in reduced soil enzyme activity, especially alkaline phosphatase, an increase in the abundance and diversity of prokaryotes, and a decline in number of eukaryotes. The community composition of less abundant bacteria (T-RFLPs comprising 0.3–1 % of total fluorescence) also shifted as a result of increased inundation under ambient salinity. Several key biogeochemical indicators (oxidation-reduction potential, CO_2 flux, porewater NH_4^+, and dissolved organic carbon) correlated with measured microbial parameters and differed with inundation level. This study indicates microbial function and composition in brackish soil is more strongly impacted by increased inundation than increased salinity. The observed divergence of microbial indicators within a short time span (10-weeks) demonstrates their usefulness as an early warning signal for shifts in coastal wetland ecosystems due to sea level rise stressors.

Keywords Coastal wetland · Peat · Sea level rise · Salinity · Mangrove

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Introduction

Much of the world's low energy and low relief coastlines are dominated by coastal wetlands, which serve a variety of ecological, economic, and social functions (e.g., habitat, coastal protection, nutrient cycling, etc.) (Gedan et al. 2009, 2011). However, the health and sustainability of these ecosystems under rising sea level remains uncertain. Predictions of coastal wetland loss due to sea level rise range from 0 to 62 % by the end of the century, depending on the region and assumptions applied (Nicholls et al. 1999; Neumann et al. 2000; Alongi 2008). In addition to the risk of wetland loss (i.e., submergence), many more coastal wetlands are likely to experience shifts in the size and distribution of plant community types (Williams et al. 1999; Ross et al. 2000; Donnelly and Bertness 2001; Teh et al. 2008). While these vegetation shifts are easily observed on the ground and remotely, significant changes in ecosystem function (e.g., biogeochemical cycling) may be

occurring long before changes in plant distribution are evident. Laboratory, mesocosm, and field manipulations investigating the impacts of sea level rise on biogeochemical processes indicate that shifts in microbial metabolic pathways, rates of C cycling, and nutrient availability occur within hours to weeks of exposure to increases in salinity and inundation (Weston et al. 2006; Chambers et al. 2011; Neubauer 2011; Chambers et al. 2013, 2014). Due to the key role soil microorganisms play in determining the balance between soil organic matter storage and mineralization (Sparling 1992), changes in their community composition, activity, or metabolic efficiency could directly impact the rate of soil accretion in coastal wetlands. Moreover, the accumulation of soil organic matter as vertical accretion determines the ability of a coastal wetland to persist and keep pace with sea level rise (Morris et al. 2002; Day et al. 2008).

Soil microbes are considered sensitive indicators of environmental change because their large surface area to volume ratio, permeable cellular membrane, and quick turnover rates result in a rapid response to variation in nutrient availability (Billings and Ziegler 2008; Ikenaga et al. 2010; Troxler et al. 2012), substrate quality (Bossio and Scow 1998; Morrissey et al. 2014a), salinity (Morrissey et al. 2014b), flooding (Mentzer et al. 2006; Unger et al. 2009), pollution (Córdova-kreylos et al. 2006), temperature (Zogg et al. 1997), and more. In this study, we sought to quantify the impact of sea level rise (i.e., the effects of increased salinity, increased inundation, and their combination) on three different soil microbial indicators: enzyme activities, phospholipid fatty acid (PLFA) signatures, and community structure (terminal restriction fragment length polymorphisms (T-RFLP)). We hypothesized that increased salinity and prolonged tidal inundation would reduce the activity of inducible soil enzymes by increasing the availability of nutrients in the soil solution. Additionally, we expected a divergence in microbial community structure based on the interactive effects of simultaneous increases in inundation and salinity would be apparent in both the PLFA and T-RFLP profiles.

Materials and Methods

Experimental Design

The study was conducted at an experimental outdoor tidal mesocosm facility located at the Florida Bay Interagency Science Center (Key Largo, FL) using 24 field replicate intact peat monoliths (25 cm deep × 25 cm diameter unvegetated soil cores) collected from a mangrove forest near Shark River Slough (SW Everglades; 25°21′52.70″ N, 81°4′40.6″ W) in August 2011. The monoliths were randomly assigned to one of four mesocosms (0.7 m D × 0.8 m W × 2.2 m L cement tubs) fed water by gravity flow from four head tanks (two

filled with freshwater (<0.5 ppt), and two with saltwater (32–40 ppt)). A system of PVC pipes and a manifold allowed for the manual mixing and delivery of the desired salinity water to each mesocosm.

The experimental design was a randomized split-plot in which salinity (the whole-plot factor) and water level (the sub-plot factor) were manipulated with 6 replicates of each condition. The "control water level" monoliths were placed on 8 cm tall risers so the soil surface was approximately halfway between low and high tide (20-cm tidal range), while the "inundated" monoliths were placed directly on the mesocosm floor. All monoliths acclimated at "ambient" salinity (~17 ppt, the in situ salinity when the soils were collected) for 3 weeks while exposed to continuous semidiurnal tides. Following acclimation, salinity was ramped-up over 7 days in two of the four mesocosms to a final "elevated" salinity of ~33 ppt. The experimental period ran for 6 weeks, during which several biogeochemical indicators were repeatedly measured (CO_2 and CH_4 flux, oxidation-reduction potential (ORP), porewater dissolved organic C (DOC) production, and porewater nutrient concentrations). A complete description of the experimental facility, study design, and biogeochemical data can be found in Chambers et al. (2014).

Soil and Porewater Analysis

At the conclusion of the study each peat monolith was destructively sampled by coring through the center with a 10 cm diameter, 25 cm long PVC core tube. The soils were divided into 3 depth segments (0–5, 5–15, and 15–25 cm). Each of the 3 depth segments was sub-divided in half; one half was refrigerated (4 °C) and the 0–5 and 5–15 cm depth segments were later analyzed for substrate quality and enzyme activities; the other half was frozen (−20 °C) and the 0–5 cm depth segment was later analyzed for PLFAs and T-RFLPs. Previously published data on soil properties (bulk density, soil moisture, % organic matter, total C, total N), porewater nutrients (NO_3^-, NO_2^-, NH_4^+, and soluble reactive phosphorus (SRP)), and DOC (Chambers et al. 2014) were used to evaluate relationships between these parameters and the microbial community characteristics presented herein.

Substrate quality for the microbial community was quantified by measuring bioavailable pools of carbon (i.e., microbial biomass C (MBC), total labile organic C (TLOC), and labile organic C (LOC)). MBC was determined by fumigation-extraction (Vance et al. 1987; White and Reddy 2001) using duplicate 5-g wet weight soil, fumigating one set with chloroform for 24 h, and extracting all samples with 25 mL of 0.5 M K_2SO_4. Samples were analyzed for DOC on a TOC 5050 A (Shimadzu Scientific Instruments, Columbia, MD) and an extraction efficiency coefficient of $k_{EC} = 0.37$ was applied (Sparling et al. 1990). TLOC was defined as the TOC for the fumigated samples (includes all OC in the soil and in the

microbial cells), LOC was defined as the TOC for the non-fumigated samples (includes OC in the soil only), and MBC was defined as the difference between TLOC and LOC (includes OC in the microbial cells only).

Enzyme Assays

Enzyme activity assays were performed within 24 h of collection on a subsample of the refrigerated soils (0–5 and 5–15 cm) using the fluorescent model substrate 4-methylumbelliferone (MUF), previously described (Chrost and Krambeck 1986; Hoppe 1993). Enzyme activities specific for C, N, P, and S substrates were assessed using β-1-4-glucosidase (BG), β-N-acetylglucosaminidase (NAG), alkaline phosphatase (AP), and aryl-sulfatase (ARS), respectively. All assays were incubated in the dark at 25 °C; AP was incubated for 2 h, while BG, NAG, and ARS were incubated for 24 h. Fluorescence was measured at excitation/emission wavelengths 360/460 on a Cytofluor 4000 multiwell plate reader (PerSeptive Biosystems, Inc.) and converted from absolute fluorescence units to nmol of active enzymes g^{-1} dry soil (Bell et al. 2013). Enzyme activity (nmol g^{-1} h^{-1}) was determined by the difference in fluorescent tag liberation between the beginning (time = 0) and the end (time = 2 h (AP), or 24 h (BG, NAG, and ARS)) of assay.

Phospholipid Fatty Acid Analysis

A subsample of the frozen surface soil (0–5 cm) from each of the 24 soil was freeze-dried and sieved to 2 mm for PLFA analysis. Analysis was performed at the University of California–Davis Analytical Laboratory following the procedure previously outlined (Bossio and Scow 1998), with the modification of only using 2 g dry wt. for extraction, which has been shown to be sufficient for highly organic soils (Jin et al. 2012). Briefly, soils were extracted for 2 h in a mixture of chloroform: ethanol: phosphate buffer (1:2:0.8 *v/v/v*). Phospholipids were separated using a solid phase extraction column (0.5 g LC-Si, Supelco, Inc.), and subjected to mild alkaline methanolysis. The resulting fatty-acid methyl esters were analyzed on a Hewlett Packard 6890 Gas Chromatograph equipped with a flame ionization detector. Peaks were identified using MIDI peak identification software (MIDI, Inc.) and calculations made using the 19:0 FAME internal standard in SAS 9.1. The nomenclature used to describe the fatty acids followed that described by Frostegård et al. (1993). The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1w5c, i17:0, a17:0, 17:0cy, 17:0, 18:1w7c, and 19:0cy were used as indicators of bacterial PLFAs, and 18:2w6,9c as an indicator of fungal PLFAs. The sum of the branched PLFAs (iso and anteiso) were used to represent Gram positive bacteria, the sum of monounsaturated PLFAs (MUFAs) represented Gram negative bacteria, and branched methylated PFLAs (10Me16:0, 10Me17:0, 10Me18:0) represented actinomycetes. PLFAs 20:4 Ω6,9,12,15c and 20:2 Ω6,9c were used to represent eukaryotes. For statistical analysis, the following key PLFA biomarkers were used: fungi, Gram positive bacteria, Gram negative bacteria, actinomycete, eukaryotes, saturated FAs, and hydroxyl FAs.

Terminal Restriction Fragment Length Polymorphisms

DNA extraction from soil samples was performed with an UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) using a subsample (0.75 g) of the 0–5 cm soil from each of the 24 soil cores. 16S rRNA genes were amplified using eubacterial universal primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′), labeled with 6′ carboxyfluorescein (6-FAM), and 1492r (5′-GTTACGACTTCACCCTCCT-3′). Polymerase chain reaction (PCR) was carried out in 100 reaction mixtures using 50 μl of Go Taq Green Master Mix (Promega Corporation, Madison, WI) according to manufacturer's instructions. Master Mix was supplemented with bovine serum albumin (48 μg/ml, final concentration). Amplification was performed by a 3-min heating step at 95 °C, followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s, with a final extension step of 10 min at 72 °C.

Following amplification, PCR products were restriction digested in separate reactions using *Msp*I and *Hha*I restriction enzymes. Reactions were performed overnight at 37 °C followed by an inactivation step at 65 °C for 10 min. T-RFLP profiles were analyzed using Peak Scanner Software v1.0 (Applied Biosystems, Foster City, CA). T-RFLPs smaller than 25 base pairs (bp) or larger than 1000 bp were excluded from the analysis. Next, profiles were exported and relative abundances were determined using the total peak height. T-RFLPs with a peak height comprising less than 0.3 % of percentage of total fluorescence (~25 fluorescence units) were considered as background noise and excluded from the analysis.

Statistical Analysis

All data was first reviewed for normality and homogeneity. BG activity was log transformed to achieve normality. Two-way MANOVAs were performed to discern overall difference in community composition according to treatment condition (salinity, inundation, and the interaction of salinity*inundation) for key biomarkers of PLFAs and enzyme activities. Where significant differences among response variables were identified with the MANOVA, univariate two-way ANOVAs and Tukey's HSD post hoc comparisons were performed to determine the nature of the relationship.

Cluster analysis was performed on the T-RFLP data using the multivariate statistical package (MVSP) version 3.12 h. The analysis and standardization of the T-RFLP profiles was conducted as described by Dunbar et al. (2001). Restriction patterns were analyzed at three different levels: (1) the entire profile (all T-RFLPs), (2) T-RFLPs comprising more than 1 % of the total fluorescence (abundant T-RFLPs), and (3) T-RFLPs comprising less than 1 % of the total fluorescence (less abundant T-RFLPs). Dendrograms were constructed using the Modified Morisita's similarity index for relative abundance data and the Jaccard's coefficient for presence/absence data. Differences in bacterial community diversity were assessed using data on both T-RFLP fragment length and proportional area to calculate community structure indices.

The Shannon-index was used to calculate diversity using all the profile data for PLFA and T-RFLP. Effects of treatment condition on these two variables were analyzed using a two-way ANOVA followed by Tukey's Multiple Comparison Test. Microbial biomass, TLOC, and LOC were also analyzed using a two-way ANOVA. Pearson's product correlation coefficients were calculated to determine relationships between enzymes, key PLFA biomarkers, and environmental variables.

Canonical Correspondence Analysis (CCA) was conducted for PLFAs and T-RFLPs to determine significant relationships between environmental variables and canonical axes according to a Monte Carlo permutation test (999 permutations) using CANOCO software (Microcomputer Power Inc., Ithaca, NY). For both analyses, the full microbial profile data was used and compared to the previously published (Chambers et al. 2014) environmental factors of: bulk density, soil moisture, % organic matter, soil total N, soil total C, TLOC, LOC, daytime CO_2 flux, nighttime CO_2 flux, and porewater concentrations of NO_3^-, NO_2^-, NH_4^+, SRP, DOC, and salinity.

Results

Substrate Quality and Enzyme Assays

MBC, TLOC, LOC, AP and ARS all decreased from 0 to 5 cm to 5–15 cm (all $p < 0.01$), whereas NAG and BG were unchanged from 0 to 15 cm. Soil enzyme activity varied significantly according to inundation treatment (two-way MANOVA, F = 3.84, $p = 0.01$), but was not affected by salinity or the interaction of salinity and inundation (F = 0.89, $p = 0.48$, and F = 1.52, $p = 0.22$, respectively). In particular, AP activity was higher in the control water level treatment (mean ± standard error, 78.49 ± 12.41 nmol g^{-1} h^{-1}) than the inundated treatment (38.89 ± 9.03 nmol g^{-1} h^{-1}, $p < 0.01$; Fig. 1). AP, NAG, and BG activities showed positive correlations with several soil properties and substrate quality
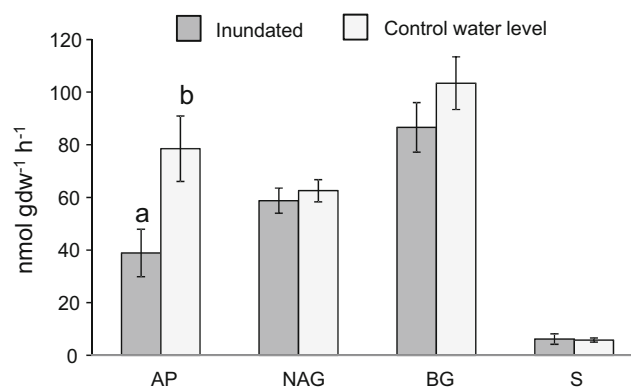

Fig. 1 Change in extracellular enzyme activity (top 0–5 cm of soil) after 10 weeks of increased tidal inundation (+8 cm), as compared to a control water level condition. AP = alkaline phosphatase, NAG = β -N-acetylglucosaminidase, BG = β-1-4-glucosidase, ARS = aryl-sulfatase. Lowercase letters denote significant differences based on a two-way ANOVA and Tukey's HSD. Error bars denote standard error

indicators (Table 1), while ARS activity showed no correlation with any measured variables. AP activity was also positively correlated with daytime ($r = 0.58$, $p < 0.01$) and nighttime ($r = 0.52$, $p = 0.01$) CO_2 flux and negatively correlated with porewater NH_4^+ ($r = -0.43$, $p = 0.04$).

Phospholipid Fatty Acid Analysis

Thirty-eight distinct PLFAs were identified in the surface soils (0–5 cm) of all 24 peat monoliths, with C chain lengths of 12–20 and total PFLA biomass of 310–558 nmol gdw^{-1}. The Shannon-index indicated higher diversity and evenness in the inundated soils (2.81 ± 0.01 and 0.76 ± 0.01, respectively) compared to the control water level soils (2.78 ± 0.01 and 0.75 ± 0.01, respectively). Community composition, as indicated by the abundance of key biomarker groups, varied

Table 1 Pearson's Product correlation coefficients (r- values above, *p*-values below in parentheses) indicating the relationship between soil enzyme activity (i.e., β-1-4-glucosidase (BG), β-N-acetylglucosaminidase (NAG), alkaline phosphatase (AP), and aryl-sulfatase (ARS)) and various soil properties (0–5 cm) in a mangrove peat soil following a 10-week mesocosm experiment

Soil Properties	AP	NAG	BG	ARS
Soil Moisture	0.00 (0.99)	**0.43 (0.04)**	0.21 (0.31)	0.27 (0.20)
% Organic Matter	0.39 (0.06)	**0.47 (0.02)**	**0.58 (<0.01)**	0.28 (0.19)
Total C	**0.41 (0.05)**	0.30 (0.15)	0.24 (0.26)	0.06 (0.77)
Total N	**0.46 (0.02)**	0.27 (0.20)	0.24 (0.25)	0.07 (0.74)
MBC	**0.46 (0.02)**	**0.57 (<0.01)**	**0.57 (<0.01)**	0.11 (0.61)
TLOC	**0.46 (0.02)**	**0.62 (<0.01)**	**0.55 (<0.01)**	0.11 (0.60)
LOC	0.16 (0.47)	0.42 (0.14)	0.11 (0.62)	0.05 (0.81)

C carbon, *N* nitrogen, *MBC* microbial biomass carbon, *TLOC* total labile organic carbon, and *LOC* labile organic carbon

Significant relationships ($p < 0.05$) are indicated in bold

significantly with inundation (two-way MANOVA, F = 4.98, p = 0.01). Univariate two-way ANOVAs indicated total PLFA (p = 0.02), Gram positive bacteria (p = 0.02), Gram negative bacteria (p = 0.01), and actinomycete (p < 0.01) had greater abundance in the inundated soils, while eukaryote biomass (p = 0.01) was greater in the control water level soils. The CCA of the PLFA data explained 56.5 % of the variation on the first two axes (Fig. 2). There was clear separation between the control water level soils and inundated soils along the first axis, which was negatively correlated with soil moisture (F = 5.15, p < 0.001). Axis 2 explained 16.6 % of the data and was positively correlated with porewater salinity (F = 2.07, p = 0.02). Pearson's correlation analysis between key PLFA biomarkers and environmental data revealed that actinomycetes abundance was positively correlated with soil moisture (r = 0.55, p = 0.04). The average porewater NH_4^+ concentration showed a positive correlation with the abundance of Gram positive bacteria (r = 0.49, p = 0.02) and Gram negative bacteria (r = 0.41, p = 0.04).

Terminal Restriction Fragment Length Polymorphisms

T-RFLP analysis revealed 760 bacterial groups (all T-RFLPs), 274 of which each comprised more than 1 % of the total fluorescence (abundant T-RFLPs) and 486 of which each comprised 0.3–1 % (less abundant T-RFLPs). Cluster analysis revealed that less abundant T-RFLPs were more affected by changes in inundation than abundant T-RFLPs. Less abundant T-RFLPs formed two distinct clusters, one in which 73 % of the soil

Table 2 Mean ± standard error (n = 12) for the abundance of key phospholipid fatty acid (PLFA) biomarkers in the surface (0–5 cm) of a mangrove peat soil exposed to simulated sea level rise conditions in a 10-week mesocosm study

Biomarker group	Control water level ($nmol\ g\ dw^{-1}$)	Increased inundation ($nmol\ g\ dw^{-1}$)
Total PLFAs*	384 ± 51[a]	442 ± 60[b]
Gram Positive**	107 ± 15[a]	126 ± 15[b]
Gram Negative*	75.5 ± 11.4[a]	90.1 ± 14.2[b]
Actinomyces**	32.4 ± 5.8[a]	39.3 ± 4.5[b]
Fungi	6.83 ± 2.1	7.99 ± 2.9
Fungi: Bacteria	0.07 ± 0.02	0.07 ± 0.02
Eukaryotes**	1.15 ± 0.5[a]	0.46 ± 0.7[b]
Saturated	119.4 ± 16.3	129.9 ± 18.9
Saturated: Unsaturated*	1.21 ± 0.10[a]	1.12 ± 0.03[b]
Cyclopropyl 17/precursor	0.46 ± 0.05	0.45 ± 0.03

Data presented according to water level treatment (i.e., control condition, and an 8-cm increase in the depth of tidal inundation)

Asterisks denote significant differences based on a two-way ANOVA and Tukey's HSD at the p < 0.05 level (*) and p < 0.01 level (**). Different letters represent significant differences (p < 0.05) based on a two-way ANOVA.

cores were from the control water level condition, and one in which 69 % of the soil core were from the inundated water level condition. This clustering was evident using both the Morisita's similarity index (relative abundance data; Fig. 3a) and Jaccard coefficient (presence/absence data; Fig. 3b).

Ambient salinity soils with increased inundation had lower diversity (Shannon-index =3.11 ± 0.20) than the control water level soils (4.09 ± 0.24; F = 4.48, p = 0.05). The CCA of the T-RFLP data explained 24.5 % of the variation on the first two axes (Fig. 4). There was clear separation of the inundated-ambient salinity soils in the bottom left quadrant. Axis 1 was negatively correlated with salinity (F = 2.14, p = 0.01) and bulk density (F = 1.74, p = 0.05), and positively correlated with TLOC (F = 2.51, p = 0.01), while axis 2 was negatively correlated with soil moisture (F = 26.6, p < 0.001). Pearson's product correlations revealed the sum of less abundant T-RFLPs was positively correlated with soil MBC (r = 0.40, p = 0.05), AP activity (r = 0.45, p = 0.03) and BG activity (r = 0.51, p = 0.01). The number of occurrences of less abundant T-RFLPs and all T-RFLPs was also positively correlated with AP activity (r = 0.43, p = 0.04) and BG activity (r = 0.47, p = 0.02).

Discussion

Microbial Community Function

Much of the existing research on saline soils demonstrates a negative correlation between enzyme activity and salinity, which is thought to be the result of, 1) direct osmotic stress interfering with enzyme synthesis (Frankenberger and Bingham 1982; Rietz and Haynes 2003; Van Ryckegem and Verbeken 2005), 2) indirect effects of salinity, such as changes in substrate quantity or quality (Neubauer 2011), or 3) changes in mineralization rate (Jackson and Vallaire 2009). However, past work has investigated freshwater soils and sediments (<0.5 ppt) when first exposed to low salt concentrations (e.g., 3–15 ppt), a range in which salinity increases are thought to have the greatest impact on the physiochemical characteristics of freshwater sediments (Rysgaard et al. 1999; Berner and Berner 2012) and cause dramatic shifts from freshwater-adapted microbial species to facultative marine species (Bouvier and del Giorgio 2002; Casamayor et al. 2002). The soils used in the present study were brackish, and already adapted to salinities above this ~15 ppt threshold, which may explain why no response to the increased salinity treatment condition was observed.

In these brackish soils, inundation, not salinity, showed the largest impact on microbial function. In particular, AP decreased under increased tidal inundation and directly correlated with CO_2 flux rates. This suggests a reduced demand for P under increased inundation (Chrost and Overbeck 1987; Sinsabaugh 1994; Wright and Reddy 2001), which may have been caused by increased P availability under more reduced

Fig. 2 Ordination results based on canonical correspondence analysis (CCA) of all PLFA biomarkers and environmental variables (TLOC = total labile organic C, LOC = labile organic C, DOC = dissolved organic C, N-CO$_2$ = nighttime CO$_2$ flux, D-CO$_2$ = daytime CO$_2$ flux). Circles drawn to assist in data interpretation of treatment effects
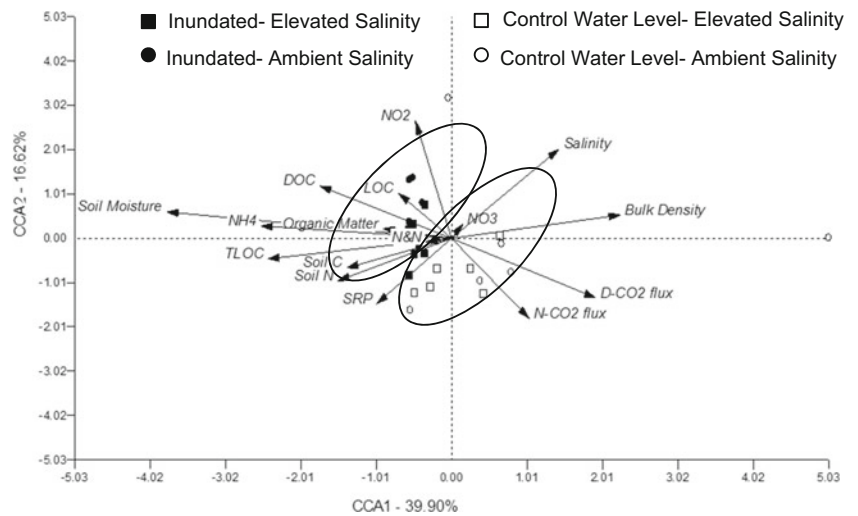
conditions, and lower rates of microbial respiration in the inundated soils (Chambers et al. 2014). Furthermore, a positive relationship between the inducible enzyme AP and CO$_2$ flux,

and the lack of such a relationship between NAG and CO$_2$ flux, could mean P availability is influencing the rate of C cycling to a greater degree than N availability.

Fig. 3 Cluster analysis of soil bacterial communities based on less abundant T-RFLPs (0.3–1 %) obtained using *Hha*I and MspI enzymes. The dendrogram was constructed by UPGMA method based on, **a** the Morisita's similarity index (relative abundance data) and, **b** Jaccard coefficient (presence/absence data), using the Multivariate Statistical Package (MVSP). Boxes drawn to indicate clustering based on treatment condition (C = Control water level, I = Inundated, A = Ambient salinity, E = Elevated salinity) and the percent of soil cores in that cluster receiving the condition indicated Alphanumeric codes refer to specific soil cores used in the experiment
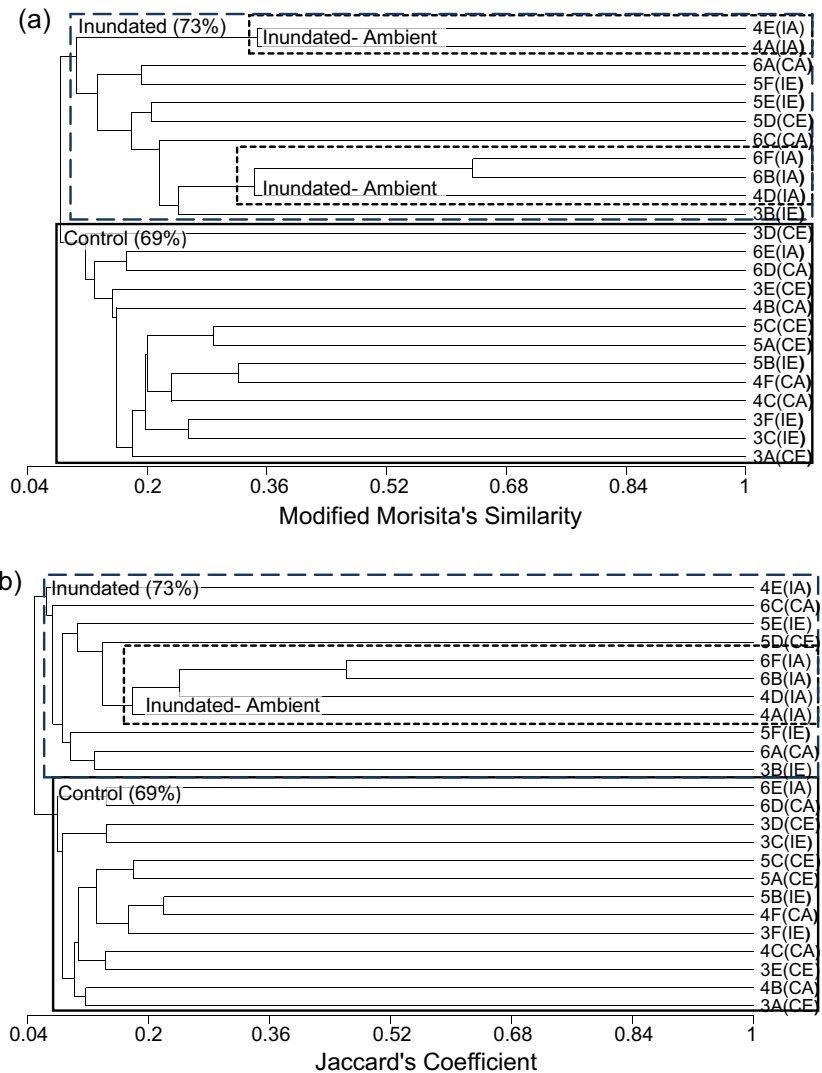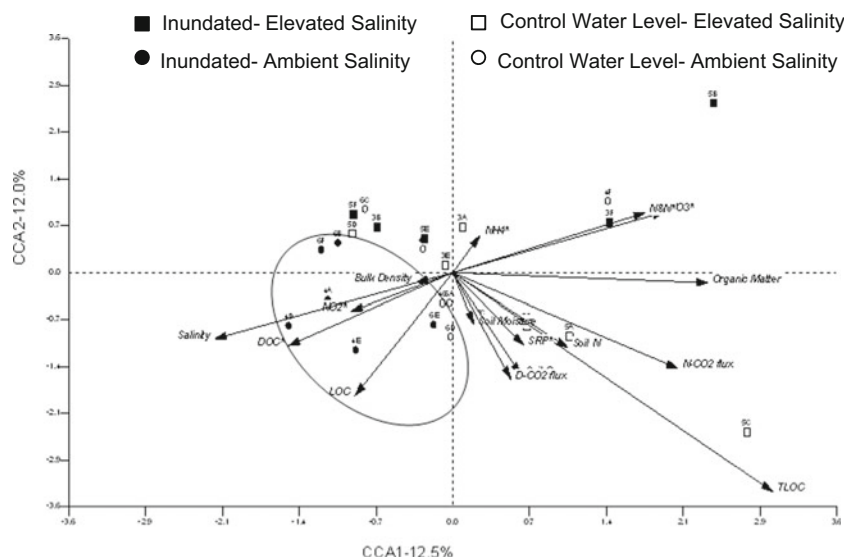
Fig. 4 Ordination results based on canonical correspondence analysis (CCA) of all T-RFLPs and environmental variables (TLOC = total labile organic C, LOC = labile organic C, DOC = dissolved organic C, N-CO$_2$ = nighttime CO$_2$ flux, D-CO$_2$ = daytime CO$_2$ flux). Circles drawn to assist in data interpretation of treatment effects

Microbial Community Composition

According to the abundance of PLFA biomarkers observed in this study, the microbial community shifted significantly in response to inundation, but was not affected by salinity, which was similar to the treatment response of enzyme activities. Previous work indicates seawater salinities higher than ~10 ppt produce conditions in which sulfate reduction is the dominant anaerobic metabolic pathway (Weston et al. 2006). Once the sulfate limitation threshold has been exceeded, additional SO$_4^{2-}$ may be unlikely to cause any major shifts in microbial community composition. On the contrary, several studies have investigated the impact of various hydrologic regimes on soil PLFA profiles and have found hydrology to be a strong predictor of microbial community structure (Córdova-kreylos et al. 2006; Mentzer et al. 2006; Unger et al. 2009; Inglett et al. 2011). A reduction in the abundance of biomarkers indicative of fungi and aerobic bacteria is often observed following flooding (Bossio and Scow 1998; Córdova-kreylos et al. 2006; Mentzer et al. 2006; Unger et al. 2009), but few other generalities regarding the nature of community shifts can be drawn from the existing literature. This study confirms the importance of hydrology in determining PLFA community structure and found that increased inundation (and lower ORP) led to greater total microbial community biomass (total PLFAs), and favored prokaryotes (Gram positive bacteria, Gram negative bacteria, and actinomycetes) at the expense of eukaryotes.

Both microbial biomass and community diversity increased with inundation, despite a general reduction in CO$_2$ flux observed in the inundated treatments during the experiment (Chambers et al. 2014). This may indicate a metabolic shift toward the production of intermediate metabolites by fermentation was initiated by a decrease in soil ORP. Limited research exists on the prevalence of fermentative

pathways in coastal wetlands due to the assumed predominance of sulfate reduction (Gribsholt and Kristensen 2003; Howarth 1984). However, intermediates such as acetate are known to accumulate in inland peat soils (Ye et al. 2014) and may still form in anaerobic microzones of saline soils (Delaune et al. 1983; Capone and Kiene 1988). Although our methods could not decipher the identity of the active microbes, the importance of fermentation in saline soils warrants further investigation.

T-RFLP analysis provided additional insight by allowing us to isolate bacteria and investigate how the treatment conditions affected groups based on their overall abundance within the community. The T-RFLP data indicated an interaction between salinity and inundation, such that the effect of increased inundation was only apparent under ambient salinity conditions. Furthermore, this shift only occurred among T-RFLPs that represented a small proportion of the overall bacterial community (comprising 0.3–1 % of the total fluorescence). This suggests increases or decreases in less common bacteria were better at defining the treatment effects on bacterial community structure than the relative abundance of all T-RFLPs or abundant T-RFLPs, which had a high degree of similarity among the four treatments. Small groups of organisms that demonstrate shifts in community structure in response to environmental gradients may be performing more specialized functions, such as methanogenesis and fermentation, while the more common groups may have greater functional redundancy (Morrissey et al. 2014a). Recent research in a variety of ecosystem types also found that rare microbial taxa make a greater contribution to temporal community dynamics than their relative abundance would suggest, especially following disturbances (Shade et al. 2014).

The differences in treatment effects found by the PLFA and T-RFLP analysis may because some of the functional groups identified in the PLFA analysis as being impacted by the main

effect of inundation were not bacteria (i.e., eukaryotes), and because T-RFLP analysis detects both viable and non-viable cells, while PLFA only detects live cells (Vestal and White 1989; Josephson et al. 1993). Using both methods together provides unique insight into the impact of the treatment conditions by demonstrating that increased tidal inundation can shift the overall microbial population (bacteria, eukaryotes and fungi), but shifts specific to the bacterial community are more prevalent among less abundant groups.

Relationship between Community Function and Structure

Linking microbial community structure to community function, whether represented by enzyme activity or biogeochemical processes, is notoriously tenuous due to the high degree of functional redundancies in soil (Nannipieri et al. 2003). In the present study, the results from both the enzyme assays and PLFA profiles emphasized the role of changes in tidal inundation (i.e., increased duration and depth of tidal flooding) in both microbial community function and structure. Furthermore, the biogeochemical data collected during this experiment (Chambers et al. 2014) also identified several key parameters that differed based on the main effect of inundation, including DOC production, porewater NH_4^+ concentration, and nighttime CO_2 flux. A reduction in soil ORP caused by increased inundation is thought to be a key factor instigating the observed divergence in PLFA biomarkers in response to inundation, as well as reducing CO_2 flux and enzyme activity (Fig. 5). An increase in abundance and diversity of specialized bacteria, possibly functioning in the production of intermediate metabolites, is suspected, but was not directly quantified in this study. The abundance and richness of less abundant T-RFLPs and all T-RFLPs were correlated with AP and BG activity, indicating a relationship between bacterial community structure and the cycling of P and C in these soils.

Experimental Considerations

Given the experimental design (i.e., soils only), we are unable to address the plant-soil feedbacks that may have interactive effects on soil ORP gradients and microbial communities. For example, live mangroves are known to transport oxygen into the root zone, increasing the surrounding soil ORP, stimulating sulfate reducers, and potentially supporting specific microbial consortia in areas proximal to roots (McKee 1993; Sherman et al. 1998). Therefore, if this study had included live vegetation, greater soil microbial community heterogeneity that was influenced by plant type and biomass, in addition to the treatment condition, would be expected. Any change in plant productivity, diversity, or litter quality are thought to cascade into shifts in microbial community function and structure (Chambers et al. 2015). Future studies should investigate the microbial community response to environmental stressors at a higher temporal resolution. Many of the functional responses quantified in this study (i.e., enzyme activity, CO_2 flux, and porewater nutrient concentrations) can respond to salinity and inundation stress within the timescale of days to weeks (Portnoy and Giblin 1997; Weston et al. 2006; Jackson and Vallaire 2009; Chambers et al. 2011, 2013). However, most studies investigating shifts in PLFA and T-RFLP profiles in soils have utilized existing environmental gradients (Córdova-kreylos et al. 2006; Ikenaga et al. 2010; Morrissey et al. 2014b) or long-term (5+ year) field manipulation experiments (Bossio and Scow 1998). Since our study ran for only 10-weeks, some findings, such as the increase in diversity of PLFAs with increased inundation, may be attributable to the samples being collected during a period of "transition" where both species adapted to the previous condition and the new treatment condition co-existed. Longer-term exposure to salinity and inundation or more frequent sampling may reveal additional shifts in community structure and function not apparent in this study (e.g., Shade et al. 2014). Future work could

Fig. 5 Conceptual diagram depicting the influence of increasing tidal inundation (+8 cm) on measured soil microbial community structural and functional properties in a mangrove peat soil exposed to increased salinity and inundation in a 10-week mesocosm experiment. An assumed mediator for the effects, though not directly quantified in this study, is represented in an oval
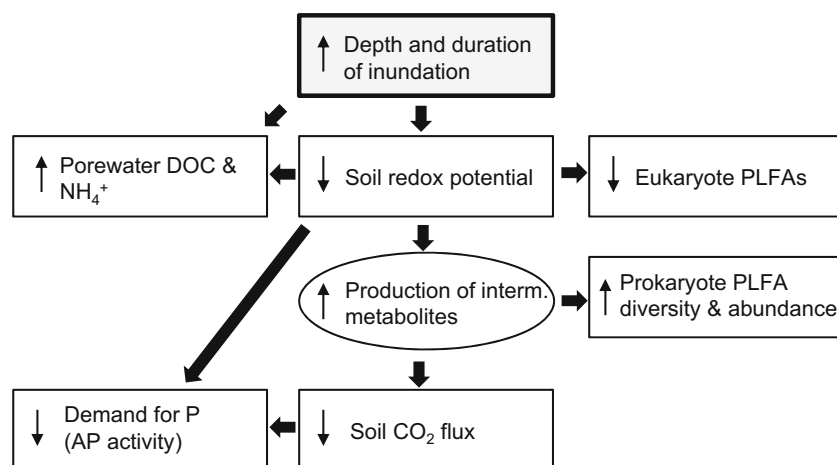
also utilize higher-resolution microbial techniques that target organisms responsible for key environmental processes (e.g., sulfate reducers, methanogens, and fermenters).

Implications for Mangroves and the Everglades

This study represents one of the first attempts to provide complementary approaches for assessing microbial community structure (PLFA and T-RFLP profiles) and function (enzyme activity, porewater chemistry, and gas fluxes) in a mangrove peat soil exposed to simulated sea level rise. While other work has provided great insight into the mechanisms and timescales with which coastal wetland biogeochemistry and microbial ecology respond to sea level rise at the freshwater end of the salinity spectrum (Weston et al. 2006; Neubauer 2011; Weston et al. 2011; Hopfensperger et al. 2014), less research has addressed the impacts of sea level rise on brackish and saline wetlands. Based on the findings of this study, increases in the depth and duration of tidal flooding may have a greater impact on the microbial community function and structure than the physiochemical changes brought about by salinity increases alone. Possibly, a positive feedback related to increased inundation may develop to encourage vertical accretion in saline wetlands- an increase in the depth of tidal inundation provides additional inorganic sediment to enhance vertical accretion and plant productivity (Fagherazzi et al. 2012; Morris et al. 2002), while also diminishing the rate of soil organic C loss (Chambers et al. 2014) and shifting the microbial community composition toward specialized species adapted to lower ORP conditions. The findings of this study emphasize the speed with which microbial indicators can respond to sea level rise stressors (i.e., within a 10-week experiment), making measurements of enzyme activity and community structure potentially useful tools for managers trying to detect early warning signs of coastal ecosystem change.

The Florida Everglades represents the largest mangrove forest in the conterminous U.S. and is highly vulnerable to sea level rise (Welch et al. 1999; Titus and Richman 2001). Studies have documented the expansion of saline marshes and mangroves at the expense of freshwater vegetation in various regions of the Everglades (Ross et al. 2000; Smith et al. 2009). Our results suggests changes in the soil microbial community are likely to precede any observed changes in plant community distribution. Identifying microbial shifts and understanding the implications of these changes on important ecosystem functions, such as soil C storage and porewater nutrient availability, could greatly improve our ability to predict the consequences of saline wetland expansion and understand the factors that contribute to coastal wetland resilience to sea level rise. Furthermore, our data demonstrates a strong relationship between the activity of AP (an enzyme that regulates P-cycling) and CO_2 flux, but no relationship the between the N-cycling enzyme NAG and CO_2 flux. This is interesting in

light of a substantial body of literature documenting the juxtaposition of the historic Everglades (a P-limited system (Davis 1991)), and the Gulf of Mexico (an N-limited system (Boyer et al. 1997; Rivera-Monroy et al. 2007)). Our results provide evidence this region of the Everglades (located ~4 km inland from the Gulf of Mexico) may still be P-limited. Understanding where the shift in limiting nutrient from P to N occurs within the coastal zone could help scientists predict how the Everglades will respond to sea level rise and associated saltwater intrusion.

References

Alongi DM (2008) Mangrove forests: resilience, protection from tsunamis, and responses to global climate change. Estuar Coast Shelf Sci 76:1–13. doi:10.1016/j.ecss.2007.08.024

Bell CW, Fricks BE, Rocca JD, et al (2013) High-throughput fluorometric measurement of potential soil extracellular enzyme activities. J Visualized Exp: JoVE e50961. doi:10.3791/50961

Berner EK, Berner RA (2012) Global environment: Water, air, and geochemical cycles. Princeton University Press, Princeton, NY

Billings SA, Ziegler SE (2008) Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO2 and N fertilization. Glob Chang Biol 14:1025–1036. doi:10.1111/j.1365-2486.2008.01562.x

Bossio DA, Scow KM (1998) Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278. doi: 10.1007/s002489900082

Bouvier TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47:453–470

Boyer JN, Fourqurean JW, Jones RD (1997) Spatial characterization of water quality in Florida Bay and Whitewater Bay by multivariate analyses: zones of similar influence. Estuaries 20:743. doi:10.2307/1352248

Capone DG, Kiene RP (1988) Comparison of microbial dynamics in marine and Freshwater sediments: contrasts in anaerobic carbon catabolism. Limnol Oceanogr 33:725–749

Casamayor EO, Massana R, Benlloch S, et al. (2002) Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ Microbiol 4:338–348. doi:10.1046/j.1462-2920.2002.00297.x

Chambers LG, Davis SE, Troxler TG, et al. (2014) Biogeochemical effects of simulated sea level rise on carbon loss in an Everglades mangrove peat soil. Hydrobiologia 726:195–211. doi:10.1007/s10750-013-1764-6

Chambers LG, Davis SE, Troxler TG (2015) Sea level rise in the Everglades: plant-soil-microbial feedbacks in response to changing physical conditions. In: Entry JA, Gottlieb AD, Jayachandrahan K,

Ogram A (eds) Microbiology of the Everglades ecosystem. CRC Press, Boca Raton, pp. 89–112

Chambers LG, Osborne TZ, Reddy KR (2013) Effect of salinity-altering pulsing events on soil organic carbon loss along an intertidal wetland gradient: a laboratory experiment. Biogeochemistry 115:363–383. doi:10.1007/s10533-013-9841-5

Chambers LG, Reddy KR, Osborne TZ (2011) Short-term response of carbon cycling to salinity pulses in a Freshwater Wetland. Soil Sci Soc Am J 75:2000–2007. doi:10.2136/sssaj2011.0026

Chrost RJ, Krambeck HJ (1986) Fluorescence correction for measurements of enzyme-activity in natural-waters using methylumbelliferyl substrates. Archiv Fur Hydrobiologie 106:79–90

Chrost RJ, Overbeck J (1987) Kinetics of alkaline-phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Plusssee (North-German eutrophic lake). Microb Ecol 13:229–248. doi:10.1007/bf02025000

Córdova-kreylos AL, Cao Y, Green PG, et al. (2006) Diversity, composition, and geographical distribution of microbial communities in California Salt Marsh Sediments Diversity, composition, and geographical distribution of microbial communities in California Salt Marsh Sediments. Appl Environ Microbiol 72:3357–3366. doi:10.1128/AEM.72.5.3357

Davis SM (1991) Growth, decomposition, and nutrient retention of Cladium-jamaicense crantz and Typha-domingensis pres in the Florida Everglades. Aquat Bot 40:203–224

Day JW, Christian RR, Boesch DM, et al. (2008) Consequences of climate change on the ecogeomorphology of coastal wetlands. Estuar Coasts 31:477–491. doi:10.1007/s12237-008-9047-6

Delaune RD, Smith CJ, Patrick WH (1983) Methane release from Gulf-coast wetlands. Tellus Ser B Chem Phys Meteorol 35:8–15

Donnelly JP, Bertness MD (2001) Rapid shoreward encroachment of salt marsh cordgrass in response to accelerated sea-level rise. Proc Natl Acad Sci U S A 98:14218–14223. doi:10.1073/pnas.251209298

Dunbar J, Ticknor LO, Kuske CR (2001) Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Appl Environ Microbiol 67:190–197

Fagherazzi S, Kirwan ML, Mudd SM, et al (2012) Numerical models of salt marsh evolution: ecological, geomorphic, and climate factors. Rev Geophys 50:28. doi:10.1029/2011rg000359

Frankenberger WT, Bingham JFT (1982) Influence of salinity on Soil enzyme activities. Soil Sci Soc Am J 46:1173–1177

Frostegård Å, Bååth E, Tunlio A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol Biochem 25:723–730. doi:10.1016/0038-0717(93)90113-P

Gedan KB, Kirwan ML, Wolanski E, et al. (2011) The present and future role of coastal wetland vegetation in protecting shorelines: answering recent challenges to the paradigm. Clim Chang 106:7–29. doi:10.1007/s10584-010-0003-7

Gedan KB, Silliman BR, Bertness MD (2009) Centuries of human-driven change in Salt Marsh Ecosystems. Ann Rev Mar Sci 1:117–141. doi:10.1146/annurev.marine.010908.163930

Gribsholt B, Kristensen E (2003) Benthic metabolism and sulfur cycling along an inundation gradient in a tidal *Spartina anglica* salt marsh. Limnol Oceanogr 48:2151–2162

Hopfensperger KN, Burgin AJ, Schoepfer VA, Helton AM (2014) Impacts of saltwater incursion on plant communities, anaerobic microbial metabolism, and resulting relationships in a restored Freshwater wetland. Ecosystems 17:792–807. doi:10.1007/s10021-014-9760-x

Hoppe H-G (1993) Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. Handbook of methods in aquatic microbial ecology 423–431

Howarth W (1984) The ecological significance of sulfur in the energy dynamics of salt marsh and coastal marine sediments. Biogeochemistry 1:5–27

Ikenaga M, Guevara R, Dean AL, et al. (2010) Changes in community structure of sediment bacteria along the Florida Coastal Everglades Marsh-Mangrove-Seagrass salinity gradient. Microb Ecol 59:284–295. doi:10.1007/s00248-009-9572-2

Inglett KS, Inglett PW, Reddy KR (2011) Soil Microbial Community composition in a restored calcareous subtropical Wetland. Soil Sci Soc Am J 75:1731–1740. doi:10.2136/sssaj2010.0424

Jackson CR, Vallaire SC (2009) Effects of salinity and nutrients on microbial assemblages in Louisiana wetland sediments. Wetlands 29:277–287

Jin XB, Huang JY, Zhou YK (2012) Impact of coastal wetland cultivation on microbial biomass, ammonia-oxidizing bacteria, gross N transformation and N2O and NO potential production. Biol Fertil Soils 48:363–369. doi:10.1007/s00374-011-0631-8

Josephson KL, Gerba CP, Pepper IL (1993) Polymerase chain-reaction detection of nonviable bacterial pathogens. Appl Environ Microbiol 59:3513–3515

McKee KL (1993) Soil physicochemical patterns and mangrove species distribution–reciprocal effects? J Ecol 81:477. doi:10.2307/2261526

Mentzer JL, Goodman RM, Balser TC (2006) Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie. Plant Soil 284:85–100. doi:10.1007/s11104-006-0032-1

Morris JT, Sundareshwar PV, Nietch CT, et al. (2002) Responses of coastal wetlands to rising sea level. Ecology 83:2869–2877

Morrissey EM, Berrier DJ, Neubauer SC, Franklin RB (2014a) Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. Biogeochemistry 117:473–490. doi:10.1007/s10533-013-9894-5

Morrissey EM, Gillespie JL, Morina JC, Franklin RB (2014b) Salinity affects microbial activity and soil organic matter content in tidal wetlands. Glob Chang Biol 20:1351–1362. doi:10.1111/gcb.12431

Nannipieri P, Ascher J, Ceccherini MT, et al. (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670. doi:10.1046/j.1351-0754.2003.0556.x

Neubauer SC (2011) Ecosystem responses of a tidal Freshwater Marsh Experiencing Saltwater intrusion and altered hydrology. Estuar Coasts 36:491–507. doi:10.1007/s12237-011-9455-x

Neumann JE, Yohe G, Nicholls RJ, Manion M (2000) Sea-level rise & global climate change: a review of impacts to U.S. coasts. 1–43

Nicholls RJ, Hoozemans FMJ, Marchand M (1999) Increasing flood risk and wetland losses due to global sea-level rise: regional and global analyses. Glob Environ Chang Hum Pol Dimens 9:S69–S87

Portnoy JW, Giblin AE (1997) Biogeochemical effects of seawater restoration to diked salt marshes. Ecol Appl 7:1054–1063. doi:10.1890/1051-0761(1997)007[1054:beosrt]2.0.co;2

Rietz DN, Haynes RJ (2003) Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol Biochem 35:845–854. doi:10.1016/s0038-0717(03)00125-1

Rivera-Monroy VH, de Mutsert K, Twilley RR, et al. (2007) Patterns of nutrient exchange in a riverine mangrove forest in the Shark River Estuary, Florida, USA. Hydrobiologia 17:169–178

Ross MS, Meeder JF, Sah JP, et al. (2000) The Southeast Saline Everglades revisited: 50 years of coastal vegetation change. J Veg Sci 11:101–112. doi:10.2307/3236781

Shade A, Jones SE, Caporaso JG, et al. (2014) Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. mBio 5:e01371–01314. doi:10.1128/mBio.01371-14

Sherman RE, Fahey TJ, Howarth RW (1998) Forest: in a neotropical interactions mangrove and sulfur dynamics iron, phosphorus. Oecologia 115:553–563. doi:10.1007/s004420050553

Sinsabaugh RL (1994) Enzymatic analysis of microbial pattern and process. Biol Fertil Soils 17:69–74. doi:10.1007/bf00418675

Smith TJ, Anderson GH, Balentine K, et al. (2009) Cumulative impacts of hurricanes on Florida mangrove ecosystems: sediment deposition, storm surges and vegetation. Wetlands 29:24–34. doi:10.1672/08-40.1

Sparling GP (1992) Ratio of microbial biomass carbon to soil organic-carbon as a sensitive indicator of changes in soil organic-matter. Aust J Soil Res 30:195–207. doi:10.1071/sr9920195

Sparling GP, Feltham CW, Reynolds J, et al. (1990) Estimation of soil microbial c by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the kec-factor. Soil Biol Biochem 22:301–307. doi:10.1016/0038-0717(90)90104-8

Teh SY, DeAngelis DL, Sternberg LDSL, et al. (2008) A simulation model for projecting changes in salinity concentrations and species dominance in the coastal margin habitats of the Everglades. Ecol Model 213:245–256. doi:10.1016/j.ecolmodel.2007.12.007

Titus JG, Richman C (2001) Maps of lands vulnerable to sea level rise: modeled elevations along the US Atlantic and Gulf coasts. Clim Res 18:205–228

Troxler TG, Ikenaga M, Scinto L, et al. (2012) Patterns of Soil bacteria and Canopy Community structure related to tropical peatland development. Wetlands 32:769–782. doi:10.1007/s13157-012-0310-z

Unger IM, Kennedy AC, Muzika RM (2009) Flooding effects on soil microbial communities. Appl Soil Ecol 42:1–8. doi:10.1016/j.apsoil.2009.01.007

Van Ryckegem G, Verbeken A (2005) Fungal diversity and community structure on Phragmites australis (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). Nova Hedwigia 80:173–197. doi:10.1127/0029-5035/2005/0080-0173

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. Soil Biol Biochem 19:703–707

Vestal JR, White DC (1989) Lipid analysis in microbial ecology- quantitative approaches to the study of microbial communties. Bioscience 39:535–541. doi:10.2307/1310976

Welch R, Madden M, Doren RF (1999) Mapping the Everglades. Photogramm Eng Remote Sens 65:163–170

Weston NB, Dixon RE, Joye SB (2006) Ramifications of increased salinity in tidal freshwater sediments: geochemistry and microbial pathways of organic matter mineralization. J Geophys Res Biogeosci 111:14. doi:10.1029/2005jg000071

Weston NB, Vile MA, Neubauer SC, Velinsky DJ (2011) Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. Biogeochemistry 102:135–151. doi:10.1007/s10533-010-9427-4

White JR, Reddy KR (2001) Influence of selected inorganic electron acceptors on organic nitrogen mineralization in everglades soils. Soil Sci Soc Am J 65:941–948

Williams K, Ewel KC, Stumpf RP, et al. (1999) Sea-level rise and coastal forest retreat on the west coast of Florida, USA. Ecology 80:2045–2063

Wright AL, Reddy KR (2001) Phosphorus loading effects on extracellular enzyme activity in everglades wetland soils. Soil Sci Soc Am J 65:588–595

Ye RZ, Jin QS, Bohannan B, et al. (2014) Homoacetogenesis: A potentially underappreciated carbon pathway in peatlands. Soil Biol Biochem 68:385–391. doi:10.1016/j.soilbio.2013.10.020

Zogg GP, Zak DR, Ringelberg DB, et al. (1997) Compositional and functional shifts in microbial communities due to soil warming. Soil Sci Soc Am J 61:475–481