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Salinity affects microbial activity and soil organic matter content in tidal wetlands

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Abstract

Climate change-associated sea level rise is expected to cause saltwater intrusion into many historically freshwater ecosystems. Of particular concern are tidal freshwater wetlands, which perform several important ecological functions including carbon sequestration. To predict the impact of saltwater intrusion in these environments, we must first gain a better understanding of how salinity regulates decomposition in natural systems. This study sampled eight tidal wetlands ranging from freshwater to oligohaline (0–2 ppt) in four rivers near the Chesapeake Bay (Virginia). To help isolate salinity effects, sites were selected to be highly similar in terms of plant community composition and tidal influence. Overall, salinity was found to be strongly negatively correlated with soil organic matter content (OM%) and C : N, but unrelated to the other studied environmental parameters (pH, redox, and above- and below-ground plant biomass). Partial correlation analysis, controlling for these environmental covariates, supported direct effects of salinity on the activity of carbon-degrading extracellular enzymes (β-1, 4-glucosidase, 1, 4-β-cellobiosidase, β-D-xylosidase, and phenol oxidase) as well as alkaline phosphatase, using a per unit OM basis. As enzyme activity is the putative rate-limiting step in decomposition, enhanced activity due to salinity increases could dramatically affect soil OM accumulation. Salinity was also found to be positively related to bacterial abundance (qPCR of the 16S *rRNA* gene) and tightly linked with community composition (T-RFLP). Furthermore, strong relationships were found between bacterial abundance and/or composition with the activity of specific enzymes (1, 4-β-cellobiosidase, arylsulfatase, alkaline phosphatase, and phenol oxidase) suggesting salinity's impact on decomposition could be due, at least in part, to its effect on the bacterial community. Together, these results indicate that salinity increases microbial decomposition rates in low salinity wetlands, and suggests that these ecosystems may experience decreased soil OM accumulation, accretion, and carbon sequestration rates even with modest levels of saltwater intrusion.

Keywords: carbon cycling, decomposition, extracellular enzyme activity, marsh, microbial community structure, saltwater intrusion, sea level rise

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Introduction

Climate change is predicted to alter the global hydrological cycle in many ways. For example, rising sea levels (Nakada & Inoue, 2005; Wigley, 2005; Church & White, 2006), reduced precipitation in watersheds (Smith *et al*., 2005) with resulting declines in stream flow (Milley *et al*., 2005), and global increases in water consumption (Gleick, 2003) may result in widespread saltwater intrusion into freshwater coastal ecosystems. Of particular concern is the impact of increased salinity on tidal freshwater wetlands, where it has been shown to drive changes in microbial metabolism (Weston *et al*., 2011; Neubauer *et al*., 2013), nutrient cycling (Weston *et al*., 2006; Marton *et al*., 2012), plant community composition (Sharpe & Baldwin, 2012), and primary production (Baldwin & Mendelssohn, 1998). Taken together, these changes may significantly alter the carbon (C) biogeochemistry and organic matter (OM) storage capacity of freshwater wetlands (Craft, 2007; Loomis & Craft, 2010). Wetlands store an estimated 45–70% of all terrestrial C (Mitra *et al*., 2005), making them important targets for conservation and major players in the global C cycle (Mcleod *et al*., 2011). One of the reasons for the high C sequestration rate of wetlands is that decomposition slows in water-saturated anaerobic soils (Reddy & DeLaune, 2008). Microbial decomposition of soil organic C and plant detritus begins with extracellular enzyme-mediated hydrolysis of complex substrates into monomers and oligomers that can be directly used for metabolism (Shi, 2011). This enzymatic hydrolysis has been proposed by many researchers to regulate decomposition rates (Sinsabaugh *et al*., 1991; Sinsabaugh & Moorhead, 1994; Schimel & Weintraub, 2003; Freeman *et al*., 2004; Allison & Vitousek, 2005) and has been tied to rates of soil respiration in multiple ecosystems including wetlands (Sinsabaugh & Findlay, 1995; Freeman *et al*., 1998; Margesin *et al*., 2000).

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Elevated salinity has been reported to both increase (Weston *et al.*, 2006; Craft, 2007), and decrease decomposition rates in wetland ecosystems (Rejmánková & Houdková, 2006; Roache *et al.*, 2006; Neubauer, 2012). These inconsistencies highlight the need for a more mechanistic understanding of how salinity affects decomposition, which can best be achieved by simultaneously studying extracellular enzymes, the proximal agents of decomposition, as well as the microorganisms responsible for enzyme production. Several prior studies have documented a salinity effect on extracellular enzyme activity (EEA) in both aquatic (Nausch *et al.*, 1998; Cunha *et al.*, 2000; Mulholland *et al.*, 2003; Neubauer *et al.*, 2013) and soil ecosystems (Rietz & Haynes, 2003; Rejmánková & Sirova, 2007). Likewise, an effect of salinity on microbial community structure has been found by scientists considering both environmental gradients (Casamayor *et al.*, 2002; Blum *et al.*, 2004; Crump *et al.*, 2004; Asghar *et al.*, 2012) and experimental manipulations (Langenheder *et al.*, 2003; Mandeel, 2006). However, very few studies have simultaneously studied the effects of salinity on microbial communities and the enzymes they produce (Rietz & Haynes, 2003; Pinckney *et al.*, 2011). This sort of information is essential if we are to understand naturally occurring patterns in soil C across estuarine systems and develop a predictive understanding of how saltwater intrusion will influence decomposition and C biogeochemistry in wetlands.

This study provides insight into the potential consequences of saltwater intrusion into freshwater ecosystems by investigating changes in microbial decomposition processes and soil OM content along a natural salinity gradient. Specifically, this work examines variation in bacterial community structure and EEA in tidal wetlands ranging from fresh to oligohaline in the Chesapeake Bay watershed (Virginia). In an attempt to isolate the effect of salinity, sampling locations were close enough in proximity that weather, land use, tidal influence, and underlying lithology were likely similar, and site selection targeted nearly identical plant communities.

Materials and methods

Site description and sampling

Samples were collected during a two week period in June 2010 from eight tidal wetlands in Virginia (Fig. 1), varying in salinity from completely fresh (ca. 0 ppt) to oligohaline (ca. 2 ppt). At each wetland, an area dominated by *Peltandra virginica* (min 75% above-ground biomass) was located and a

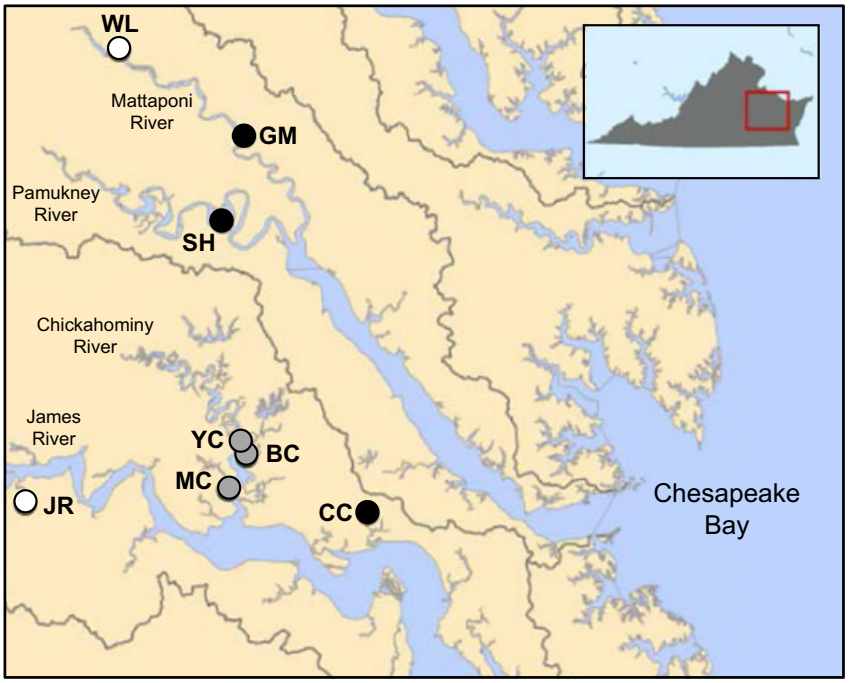

Fig. 1 Map of sampling locations along four tidal rivers proximal to the Chesapeake Bay (Virginia). The lowest salinity sites (white symbols, ppt < 0.1) are Walkerton Landing (WL) and James River National Wildlife Refuge (JR). Intermediate salinity sites (gray symbols, 0.1 < ppt < 0.4) are Yarmouth Creek (YC), Blackstump Creek (BC), and Morris Creek (MC). The most saline sites (black symbols, ppt > 0.4 are Gleason Marsh (GM), Sweet Hall Marsh (SH), and College Creek (CC).

10×10 m^2 plot was established. Within each plot, five sampling stations (1 m^2 each) were randomly selected with the caveat that the minimum separation distance between stations was 3 m. All living plant material found within each 1 m^2 area was harvested to determine above-ground biomass (AGB, clipped to the soil surface). In addition, a 60 cm^3 plastic syringe was modified to form a miniature soil corer and used to collect soil for quantifying below-ground biomass (BGB). Lastly, samples of surface soil (0–10 cm) were placed in airtight plastic bags and returned to the laboratory on ice, at which point each sample was homogenized and subdivided for analysis of soil properties, enzyme activity (ca. 50 g stored at 4 °C), and bacterial community structure (ca. 5 g stored at −20 °C).

Soil properties

In the laboratory, soil was immediately analyzed for pH and redox potential (Hanna Combo pH and ORP probe, Smithfield, RI, USA), OM concentration (% as loss on ignition, 425 °C for 12 h), and C and N content (Perkin Elmer Series II CHNS/O Analyzer 2400; Waltham, MA, USA). In addition, porewater was collected from 5-ml soil samples by centrifugation (3000 g for 15 min), filtered using a 0.45 μm pore size mixed cellulose ester syringe filter, and stored at −20 °C until it could be analyzed for the concentration of chloride via ion chromatography (Dionex ICS-1000, Sunnyvale CA, USA). Salinity (ppt) was then calculated as described by Bianchi (2006).

Plant biomass

The plant material harvested from each station was dried (70 °C for 7 days) to estimate AGB as kg of dry plant material per m^2. BGB was determined by submerging each relevant soil core in 35 ml of tap water (24 h) prior to straining through a No. 45 metal mesh (U.S.A. Standard Testing Sieve, A.S.T.M. E-11 specification, Fisher Scientific, Waltham MA, USA). Roots were collected by hand, dried (95 °C for 96 h), and BGB was calculated as mg of dry roots per cm^3 of soil.

Enzyme activity

Rates of EEA were determined within 1 week of sampling using the fluorimetric and colormetric microplate assays described in Neubauer *et al.* (2013) and a Synergy 2 plate reader (Biotek, Winooski, VT, USA). Briefly, enzyme activity associated with breakdown of cellulose [β-1,4-glucosidase (BG) and 1,4- β-cellobiosidase (CBH)], hemicellulose [β-D-xylosidase (BX)], and lignin [phenol oxidase (POX)], as well as the release of phosphorus [alkaline phosphatase (AP)] and sulfur [arylsulfatase (AS)] from organic molecules, was measured. All substrates and reagents were obtained from Sigma-Aldrich Co. Ltd (St. Louis, MO, USA). Methylumbelliferone (MUB)-linked assays relied on the following substrates: 4-MUB β-D-glucopyranoside (BG), 4-MUB β-D-cellobioside (CBH), 4-MUB- β-D-xylopyranoside (BX), 4-MUB-phosphate (AP), and 4-MUB-sulfate (AS) and incubations of either 1 (CBH, AP) or 4 h (BG,BX, AS) at 30 °C with gentle agitation. POX activity was measured colorimetrically via the oxidation of l-DOPA after a 30 min incubation using the methods of Sinsabaugh *et al.* (2003).

Bacterial abundance

Whole-community DNA was extracted from 0.25 g subsamples of soil using the Mo Bio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) and then stored at −20 °C. DNA purity and concentration were analyzed using a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). All DNA extracts and PCR products were verified using agarose gel (1.5%) electrophoresis and ethidium bromide staining.

Bacterial abundance was estimated using the quantitative polymerase chain reaction (qPCR) approach outlined in Fierer *et al.* (2005); specifically, we targeted the *16S rRNA* gene using the Eub338 and Eub517 primers and analytical details presented in Morrissey *et al.* (2013). Assays utilized SsoAdvanced SYBR Green qPCR Supermix (BioRad, Hercules, CA, USA) and the BioRad CFX 96 Real-Time System; data were analyzed using Bio-Rad CFX Manager Version 2.1. Samples were run in triplicate and averaged; standard curves were established using genomic DNA from *Escherichia coli* (Strain #11775, ATCC, Manassas, VA, USA; average efficiency = 101%, $r^2 = 0.99$).

Bacterial community structure

Bacterial community structure was analyzed using terminal restriction fragment length polymorphism (T-RFLP) targeting the *16S rRNA* gene of bacteria and the protocols described in Morrissey *et al.* (2013). Briefly, PCRs included 1.2 ng of template DNA and the domain-specific primers 27F (labeled with FAM) and 1492R at a concentration of 0.2 μM each. PCR products were digested using Hha1 (New England Biolabs, Ipswich, MA, USA) and detected via capillary electrophoresis using a MegaBACE 1000 DNA Analysis System (Amersham Biosciences, Buckinghamshire, UK). T-RFLP fragments between 70 and 400 base pairs (bp) were analyzed using Fragment Profiler software (Version 1.2; Amersham Biosciences, Buckinghamshire, UK) with a 1-bp size differential and a 40 relative fluorescent unit peak height threshold. The T-RFLP results were recorded as a binary data matrix describing the presence or absence of each terminal restriction fragment in each sample.

Statistical analyses

Shapiro–Wilks tests confirmed that soil pH, redox potential (mV), AGB (kg m^{-2}), and BGB (mg cm^{-3}) data were normally distributed. A natural log (ln) transformation was necessary for salinity (ppt), all enzyme activity measures (μmoles g-OM^{-1} h^{-1}), and bacterial abundance (copies g-OM^{-1}); transformed values were utilized in all subsequent statistical analyses. Site differences were analyzed using one-way analysis of variance (ANOVA; $n = 5$, $df = 39$, $\alpha = 0.05$) with Tukey's HSD for post hoc comparisons. Direct and

partial correlations (Pearson's r, $n = 40$) were used to evaluate relationships between bacterial abundance, enzyme activity, and environmental variables; statistical significance was established using a sequential Holm–Bonferroni adjustment to correct for multiple comparisons (Holm, 1979). All ANOVAS and Pearson's correlations were performed using the JMP statistical software (Version JMP Pro 9.0.2, Cary, NC, USA; Sall, 2005).

The binary matrix of T-RFLP data was converted to a set of Jaccard coefficients, which were used for all statistical analyses that considered bacterial community composition. Visualization of overall similarity between samples was achieved using nonmetric multidimensional scaling (NMDS, 2-dimensional solution) and site differences were analyzed using one-way nonparametric multivariate analysis of variance (NP-MANOVA; $n = 5$, $df = 39$, $\alpha = 0.05$). Mantel and partial Mantel tests (r_M) were performed to compare the changes in bacterial community composition (Jaccard similarity matrix) with the changes in environmental and enzyme variables (Euclidian distance matrices) All community composition analyses were performed in PAST statistical software package (Version 2.10; Hammer, 2001).

Results

Site differences

Sites varied significantly with respect to porewater salinity, from fresh (0.03 ppt at JR) to brackish (1.85 ppt at CC, Table 1). Likewise, soil characteristics (pH, redox, %OM, and C : N) and macrophyte biomass (AGB and BGB) exhibited significant differences between sites, though all parameters were within the natural ranges expected for these types of wetlands (Reddy & DeLaune, 2008; Barendregt *et al.*, 2009).

The activity of all enzymes varied significantly across sites (Table 2, rates in μmoles g-OM^{-1} h^{-1}). For the hydrolytic carbon-degrading enzymes (CBH, BG, and BX), rates were lowest at JR (CBH = 0.13, BX = 0.03) and/or WL (BG = 0.09, BX = 0.03) and highest (ca. 5-times greater) at SH (mean: CBH = 0.75, BG = 0.36, and BX = 0.12). This pattern was also observed for POX [lowest at JR (mean: 31) and highest at SH (mean: 1071); ca. 35-fold increase] and AP [lowest at JR (mean: 0.22) and highest at SH and GM (mean: 1.39); ca. sixfold increase]. Activity of AS also varied ~sixfold from the site with the lowest activity (BC=0.08) to the site with the highest activity (YC = 0.45).

Bacterial abundance differed significantly across sites (Table 1), ranging from the lowest at BC (8.3×10^9 copies g-OM^{-1}) to the highest at GM (35.3×10^9 copies g-OM^{-1}, ca. 4-times greater). The community structure of bacteria also differed across sites (NP-MANOVA $F = 7.32$, $P < 0.01$); all pairwise comparisons between sites yielded $P < 0.03$.

Table 1 Site locations and mean (±SE) of environmental parameters for each site. Values with the same superscript letter are not significantly different from each other (ANOVA and Tukey's HSD post hoc test with $\alpha = 0.05$; salinity and bacterial abundance were natural log (ln) transformed values prior to testing)

				Soil properties				Plant biomass		
Site	GPS (N)	GPS (W)	Porewater salinity (ppt)	pH	Redox (mV)	OM (%)	C : N	AGB* (kg m^{-2})	BGB†(mg cm^{-3})	Bacterial abundance ($\times 10^9$ copies g-OM^{-1})
JR	37°16′27″	77°09′18″	0.03 ± 0.01^{a}	6.1 ± 0.1^{ab}	−11 ± 5^{b}	37.4 ± 2.9^{a}	12.7 ± 0.6^{ab}	0.23 ± 0.04^{b}	4.8 ± 1.1^{b}	13.5 ± 2.6^{ab}
WL	37°43′60″	77°00′96″	0.04 ± 0.01^{a}	5.6 ± 0.2^{b}	309 ± 26^{a}	35.8 ± 1.7^{a}	12.6 ± 0.5^{ab}	0.19 ± 0.04^{b}	4.4 ± 1.8^{b}	12.4 ± 2.6^{ab}
YC	37°19′64″	76°52′26″	0.15 ± 0.03^{b}	6.4 ± 0.1^{a}	−96 ± 9^{b}	10.4 ± 2.0^{b}	11.4 ± 0.2^{ab}	0.16 ± 0.02^{a}	23.8 ± 3.4^{b}	23.7 ± 2.8^{bc}
MC	37°16′78″	76°53′38″	0.16 ± 0.01^{b}	6.2 ± 0.2^{ab}	−65 ± 28^{b}	18.2 ± 0.6^{b}	11.5 ± 0.3^{ab}	0.12 ± 0.03^{b}	6.34 ± 0.3^{b}	18.9 ± 2.8^{abc}
BC	37°18′76″	76°51′89″	0.17 ± 0.02^{b}	6.2 ± 0.1^{ab}	23 ± 19^{b}	34.2 ± 5.0^{a}	12.9 ± 0.6^{a}	0.11 ± 0.03^{a}	30.0 ± 3.4^{b}	8.3 ± 2.1^{a}
GM	37°38′14″	76°51′39″	0.54 ± 0.04^{c}	5.6 ± 0.1^{a}	255 ± 58^{a}	15.3 ± 0.4^{b}	10.8 ± 0.4^{b}	0.53 ± 0.12^{b}	1.0 ± 0.2^{a}	35.3 ± 8.9^{c}
SH	37°33′02″	76°53′31″	0.88 ± 0.08^{cd}	6.2 ± 0.2^{ab}	−64 ± 31^{b}	14.2 ± 0.6^{b}	11.3 ± 0.3^{ab}	0.14 ± 0.05^{b}	6.1 ± 2.2^{b}	28.2 ± 4.3^{bc}
CC	37°15′08″	76°42′60″	1.85 ± 0.11^{d}	6.4 ± 0.2^{a}	−73 ± 22^{b}	16.4 ± 0.7^{b}	10.8 ± 0.7^{b}	0.20 ± 0.04^{b}	9.1 ± 2.7^{b}	28.2 ± 4.6^{bc}

*Above-ground biomass.
†Below-ground biomass.

Table 2 Mean (±SE) enzyme activity rates for each site. Values with the same superscript letter are not significantly different from each other (ANOVA and Tukey's HSD post hoc test on natural log (ln) transformed values, $\alpha = 0.05$)

	Enzyme Activity (μmoles g-OM^{-1} h^{-1})					
Site	CBH	BG	BX	POX	AP	AS
JR	0.13 ± 0.01^{a}	0.11 ± 0.02^{ab}	0.03 ± 0.01^{a}	31 ± 9^{a}	0.22 ± 0.03^{a}	0.10 ± 0.02^{ab}
WL	0.15 ± 0.01^{a}	0.09 ± 0.01^{a}	0.03 ± 0.00^{a}	100 ± 20^{b}	0.31 ± 0.03^{a}	0.09 ± 0.01^{ab}
YC	0.46 ± 0.01^{bc}	0.28 ± 0.04^{c}	0.10 ± 0.01^{b}	365 ± 26^{c}	0.87 ± 0.04^{b}	0.45 ± 0.00^{c}
MC	0.41 ± 0.03^{b}	0.18 ± 0.03^{abc}	0.09 ± 0.01^{b}	222 ± 39^{bc}	0.84 ± 0.07^{b}	0.24 ± 0.04^{bc}
BC	0.20 ± 0.05^{a}	0.25 ± 0.06^{bc}	0.07 ± 0.01^{b}	295 ± 101^{b}	0.36 ± 0.09^{a}	0.08 ± 0.04^{a}
GM	0.61 ± 0.03^{bc}	0.26 ± 0.03^{c}	0.09 ± 0.01^{b}	501 ± 74^{cd}	1.39 ± 0.05^{b}	0.34 ± 0.02^{c}
SH	0.75 ± 0.08^{c}	0.36 ± 0.05^{c}	0.12 ± 0.02^{b}	1071 ± 238^{d}	1.39 ± 0.13^{b}	0.40 ± 0.04^{c}
CC	0.50 ± 0.06^{bc}	0.23 ± 0.02^{bc}	0.09 ± 0.01^{b}	250 ± 39^{b}	1.10 ± 0.12^{b}	0.36 ± 0.04^{c}

Correlation analysis

Salinity. Salinity was negatively correlated with soil OM content ($r = -0.65$, $P < 0.01$) and C:N ($r = -0.50$, $P < 0.01$, Fig. 2), but unrelated to the other environmental variables (all $|r| < 0.29$, $P > 0.07$). Salinity exhibited strong positive correlations with EEA (Fig. 3; all $r > 0.58$, all $P < 0.01$) and bacterial abundance (Fig. 4; $r = 0.56$, $P < 0.01$). Similarly, salinity was the strongest environmental correlate with bacteria community structure ($r_M = 0.32$, $P < 0.01$). This relationship can be visualized on the lower panel of Fig. 4 where increasing salinity is associated with a positive shift on the NMDS Axis 1, and a negative shift on Axis 2. This is reflected by a salinity vector that represents the strength and direction of the correlation of salinity with each NMDS axis.

Soil OM. In addition to the relationship with salinity, soil OM was strongly correlated with C : N ratio ($r = 0.67$, $P < 0.01$), but unrelated to the other environmental parameters (all $|r| < 0.26$, $P > 0.09$). Furthermore, OM was negatively correlated with enzyme activity rates (Pearson's r: CBH = −0.91, BG = −0.65, BX = −0.80, POX = −0.73, AP = −0.90, AS = −0.91; all $P < 0.01$) as well as bacterial abundance ($r = -0.64$, $P < 0.01$). Changes in soil OM were not significantly related to changes in bacterial community composition following the Holm's Bonferroni correction ($r_M = 0.13$, $P = 0.04$).

Other environmental variables. The only other significant relationships among environmental variables were between redox and pH ($r = -0.73$, $P < 0.01$) and between AGB and both pH ($r = -0.47$, $P < 0.01$) and redox ($r = 0.52$, $P < 0.01$). In addition to salinity and OM (discussed above), EEA was correlated with C : N (Pearson's r: CBH = −0.65, BG = −0.42, BX = −0.46, POX = −0.48, AP = −0.71, AS = −0.74; all $P < 0.01$), but unrelated to all other measured environmental variables. The only other significant correlation for bacterial abundance was with AGB ($r = 0.46$, $P < 0.01$). Bacteria community structure was unrelated to all other environmental variables (all $r_M < 0.12$ and $P > 0.05$). Full correlation results are presented in Table S1.

Partial correlation analysis

To determine the extent to which salinity was a direct driver of EEA and the bacterial community, a partial correlation analysis was performed to control for the main environmental covariates (%OM and C : N; Table 3). All enzymes remained significantly correlated with salinity after controlling for C : N ($r > 0.35$, $P < 0.04$); similarly, relationships remained significant after controlling for OM ($r > 0.32$, $P < 0.05$) except for AS rates. Bacterial abundance remained significantly correlated with salinity after controlling for C : N, but not OM. The association between salinity and community structure was robust and remained highly significant after controlling for covariates with salinity (both $r_M > 0.29$ with $P < 0.01$).

Relationships between the bacterial community and EEA were also examined. Bacterial abundance was significantly correlated with all enzymes except BG, and three of these relationships remained significant after controlling for covariation with salinity via partial correlation (Table 4). Furthermore, the composition of the bacterial community was significantly related to CBH, POX, and AP, even following a partial Mantel test to account for any shared relationship with salinity.

Discussion

The aim of this study was to examine changes in bacterial community structure and function (EEA) along a salinity gradient from fresh to oligohaline to better understand how salinity regulates soil OM

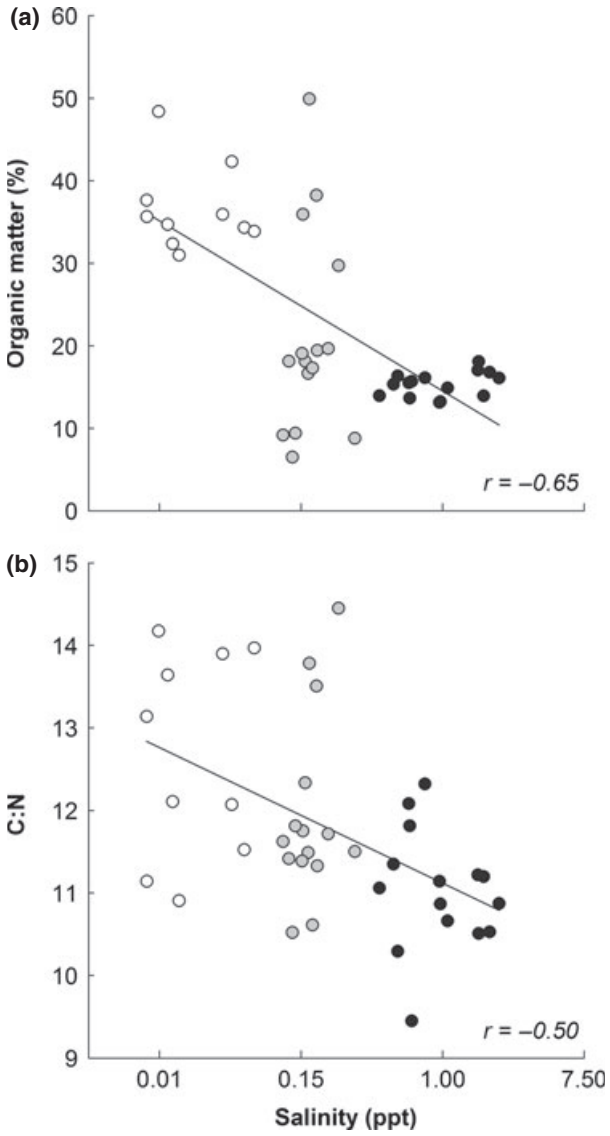

Fig. 2 Variation in (a) organic matter and (b) C : N with salinity [natural log (ln) scale]. Correlation results are shown in the lower right corner (Pearson's *r*, both $P < 0.01$). Data points are colored by salinity (white < 0.1 ppt, gray = 0.1 < ppt < 0.4, and black > 0.4 ppt).

decomposition and storage in wetlands. The role of salinity as a driver of ecosystem processes in tidal freshwater wetlands is particularly important in light of the potential for climate change-associated saltwater intrusion. Generally, there have been few studies that focus on this range of salinity (Poffenbarger *et al.*, 2011; Neubauer, 2012; Sharpe & Baldwin, 2013), even though the transition from fresh to oligohaline is what many historically freshwater systems are likely to experience as a consequence of sea level rise in the coming decades (Woodroffe & Murray-Wallace, 2012).

The negative relationship between soil OM and salinity exhibited in the current work is consistent with Craft (2007), who found soil organic C (%) to be negatively related to salinity in tidal wetlands across the conterminous United States. Specifically, for the 63 studies surveyed in Craft's work, soil bulk density and organic C content were consistently lower in saltier marshes than their freshwater counterparts regardless of geographical region. We consider three nonexclusive possible explanations for this pattern: (i) enhanced tidal influences that increase OM export and/or sediment deposition in saltier marshes; (ii) decreased plant production and/or altered community composition, which changes the quality and quantity of OM deposited on the marsh surface; and (iii) increased decomposition rates at more saline sites.

Tidal range has been shown to be a strong regulator of aqueous export of total organic C and suspended solids from marsh ecosystems (Childers *et al.*, 2000) and could potentially affect mineral sediment deposition (Chmura & Hung, 2004). Consequently, a greater tidal range at the more saline wetlands could contribute to the lower levels of soil OM at these sites. Although not directly measured in this study, we expect the differences in tidal range across the sites we sampled were not sufficient to explain the OM patterns. For instance, tidal range predictions by NOAA on the James River (20 year average from http://tidesandcurrents.noaa.gov) indicate that the mean tidal range near our most saline site is only a few inches greater than for the most upriver freshwater site (2.26 vs. 2.15 ft). In addition, soil texture (determine by the hydrometer method using one aggregate sample per site; results not presented) did not correlate with salinity (considering % sand, silt, and clay, all $P > 0.29$), which suggests no consistent changes in sediment deposition along the salinity gradient of our sites.

The second explanation of the salinity-OM relationship we observed – that is, the effect of saltwater on plant production and/or community composition – was also not well supported. We saw no correlation between above- or below-ground plant biomass and either salinity or soil OM content, and plant production differed little across sites (Table 1). This is likely due to the relatively narrow salinity range we considered, as prior work has shown decreased plant productivity (McKee & Mendelssohn, 1989; Więski *et al.*, 2010) as well as differences in below-ground biomass allocation (Neubauer *et al.*, 2005) along broader salinity gradients. Aside from biomass production, salinity could influence plant community composition and diversity (Latham *et al.*, 1994; Więski *et al.*, 2010; Tuxen *et al.*, 2011). Because various plant species differ in polymer composition (Kögel-Knabner, 2002), this could affect the

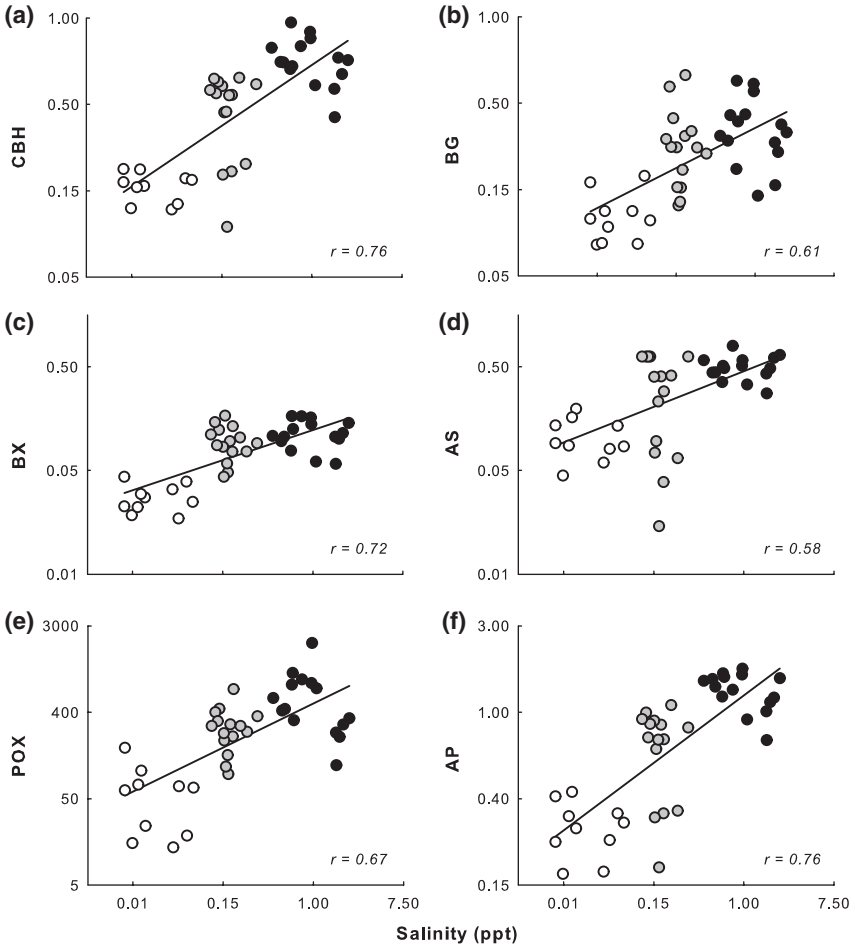

Fig. 3 Variation in extracellular enzyme activity (rates in μmoles g-OM^{-1} h^{-1}) with salinity; both parameters are presented using a natural log (ln) scale. Correlations results are shown in the lower right corner of each panel (Pearson's r, all $P < 0.01$). Data points are colored by salinity (white < 0.1 ppt, gray = 0.1 < ppt < 0.4, and black > 0.4 ppt). Enzyme abbreviations are as follows: (a) CBH = 1, 4-β-cellobiosidase, (b) BG = β-1, 4-glucosidase, (c) BX = β-D-xylosidase, (d) AS = arylsulfatase, (e) POX = phenol oxidase, and (f) AP = alkaline phosphatase.

quality of the litter deposited on the marsh surface and potentially alter rates of OM accumulation. While this possibility cannot be completely ruled out in our study, a large variation in litter quality is not expected due to the fact that site selection targeted plots that were dominated by a single perennial species (*Peltandra virginica*).

Given the similar tidal regimes and plant communities at each of our sites, the third of our possible explanations for the salinity-OM relationship – increased decomposition under more saline conditions – is best supported by our results. In particular, we documented strong relationships between salinity and enzyme activity (Fig. 3), soil OM content (Fig. 2), and microbial community structure (Fig. 4) that typify enhanced decomposition. This could result from a combination of factors whereby salinity: (i) increases the bioavailability of organic substrates; (ii) facilitates enzyme activity; and (iii) stimulates changes in microbial community structure. Furthermore, we propose that salinity's influence in this study derives largely from the effect of increased ionic strength on the molecular stability and sorption of both enzymes and their organic substrates. With regard to OM, increased ionic strength can make organic particles more accessible by disrupting soil microaggregates (Rengasamy & Sumner, 1998) and increasing the number of macropore spaces in the soil profile (Brady & Weil, 2004) potentially affecting soil aeration (Blackwell *et al.*, 1990; Kühne *et al.*, 2012). Ionic strength could also affect solute concentrations of organic compounds by decreasing their sorption to soil particles (Reemtsma *et al.*, 1999; Mavi & Marschner, 2012). Both these processes would increase microbial access to organic substrates and, as past studies have found enzyme activity rates to be positively related to

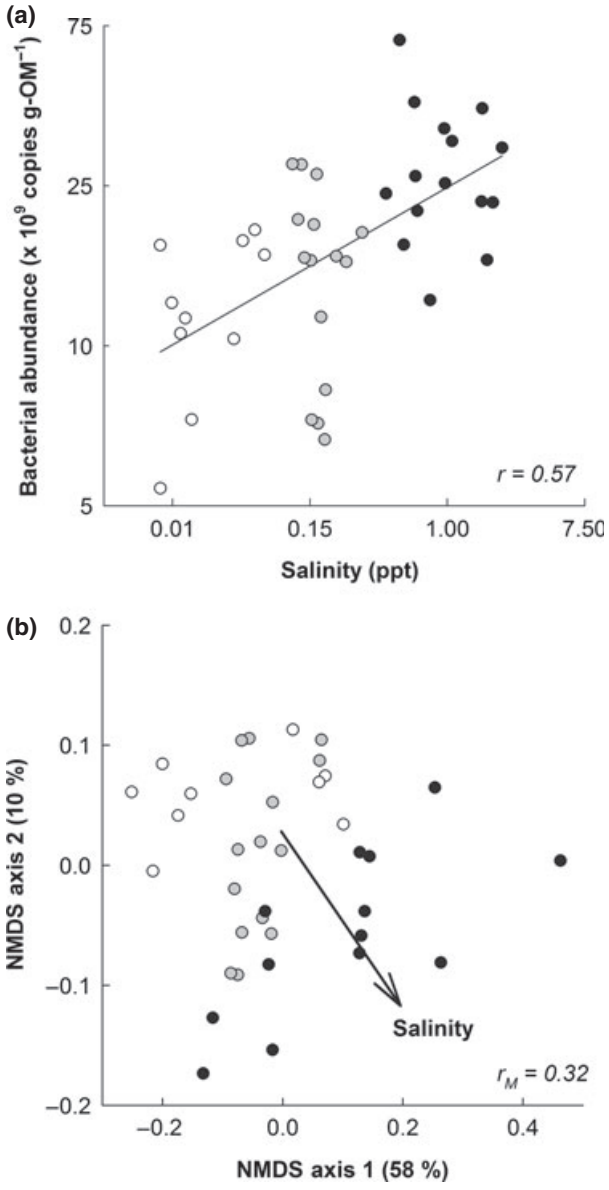

Fig. 4 Variation in (a) bacterial abundance and (b) community composition with salinity. Bacterial abundance and salinity axes use a natural log (ln) scale. Community composition is displayed using an NMDS ordination diagram (stress = 0.20) wherein the vector presents the relationship of salinity with each axis. Correlation results for salinity with abundance (Pearson's, *r*) and composition (Mantel test, r_M) are shown in the lower right corner. Data points are colored by salinity (white < 0.1 ppt, gray = 0.1 < ppt < 0.4, and black > 0.4 ppt).

target molecule availability (Shackle *et al.*, 2000; Allison & Vitousek, 2005; Morrissey *et al.*, 2013), this could explain the higher levels of enzyme activity we observed at more saline sites (Fig. 3). Similarly, the abundance of cations in seawater can affect inorganic N exchange and P sorption (Portnoy & Giblin, 1997; Weston *et al.*, 2006, 2011), allowing higher salinity to liberate otherwise limiting nutrients (Weston *et al.*, 2010). It is important to point out that the electrostatic interactions described above are interactively affected by other environmental parameters (e.g., pH) and soil physicochemical properties like surface area. For example, increased pH can limit the binding capacity of clay compounds, leading to decreased humic acid sorption (Vermeer *et al.*, 1998; Abate & Masini, 2003). All these effects are also somewhat dependent on the chemical nature of the C compounds involved. In particular, the behavior of humic substances, which constitutes 50–80% of the natural OM (Shaker *et al.*, 2012), will be governed by the relative abundance of functional groups like carboxyls, hydroxyls, and phenolics.

Besides ionic strength effects on organic substrate availability, it is also necessary to consider how changes in salt concentration may directly impact extracellular enzymes. With regard to sorption, several studies have documented a decrease in mobility and catalytic activity when enzymes are adsorpted to clay surfaces (Quiquampoix, 1987; Fusi *et al.*, 1989; Tietjen & Wetzel, 2003), and such immobilization reduces the probability of contact between enzyme and substrate (Lammirato *et al.*, 2010). However, scientists' understanding of how these proteins interact with mineral surfaces is limited, especially when considering confounding factors such as solution pH and enzyme molecular weight. As it appears that the majority of extracellular enzymes are adsorbed to soil particles (Kandeler, 1990; Gianfreda & Bollag, 1994; Violante *et al.*, 1995; Lipson & Nasholm, 2001; Nannipieri *et al.*, 2003), this is an essential area for

Table 3 Partial correlation analysis comparing salinity to enzyme activity (Pearson's *r*), bacterial abundance (Pearson's *r*), and bacterial community composition (Mantel test, r_M) while controlling for soil OM and C : N

	Partial correlation controlling for			
	OM		C : N	
	r	*P*	*r*	*P*
Enzyme				
CBH	0.52	<0.01*	0.65	<0.01*
BG	0.33	0.04*	0.51	<0.01*
BX	0.44	<0.01*	0.64	<0.01*
POX	0.38	0.02*	0.57	<0.01*
AP	0.55	<0.01*	0.68	<0.01*
AS	−0.05	0.74	0.36	0.03*
Bacteria				
Abundance	0.26	0.11	0.48	<0.01*
Composition	0.30	<0.01*	0.32	<0.01*

*$P < 0.05$.

Table 4 Direct and partial (controlling for salinity) correlation analysis comparing enzyme activity to bacterial abundance (Pearson's r) and community composition (Mantel test, r_M)

	Bacterial abundance				Bacteria Composition			
	Direct		Partial		Direct		Partial	
Enzyme	r	P	r	P	r_M	P	r_M	P
CBH	0.62	<0.01*	0.34	0.03*	0.26	<0.01*	0.14	0.02*
BG	0.31	0.05	−0.05	0.75	0.09	0.09	−0.01	0.52
BX	0.44	<0.01*	0.06	0.73	0.11	0.06	−0.04	0.73
POX	0.39	0.01*	<0.01	0.98	0.42	<0.01*	0.35	<0.01*
AP	0.63	<0.01*	0.36	0.02*	0.27	<0.01*	0.14	0.02*
AS	0.60	<0.01*	0.41	0.01*	0.10	0.08	0.03	0.25

*$P < 0.05$.

future research if we are to develop a predictive understanding of how C mineralization is affected by saltwater intrusion.

Changes in salinity also have the potential to impact extracellular enzyme activity by influencing molecular stability and protein confirmation states. Most laboratory studies show a detrimental effect of salt on enzyme activity, but these assays use purified enzymes in solution and only a select group of substrates (Das *et al.*, 1997; Fang *et al.*, 2010). Work in soils similarly shows high salt concentrations inhibit enzyme activity, but these studies target hypersaline conditions well beyond the range relevant to our study (e.g., Tripathi *et al.*, 2007; Yun *et al.*, 2010; Pan *et al.*, 2013). The limited reports from wetland soils are inconsistent. Even within a single study, results vary by enzyme, and there is evidence that activity can simultaneously be enhanced, suppressed, and unaffected by salinity (Wu *et al.*, 2008; Jackson & Vallaire, 2009; Saviozzi *et al.*, 2011; Chambers *et al.*, 2013; Neubauer *et al.*, 2013). There are several noteworthy differences between the studies cited above and our own, in which we found salinity significantly enhanced enzyme activity for a diversity of substrates (e.g., breakdown of both labile and recalcitrant compounds, acquisition of C, N, and P). First, the research efforts differed dramatically in their potential for plant effects. Our experimental design minimized the influence of salinity-induced differences in plant biomass and community composition, which was a significant covariant in much of the prior work. Second, our study compared sites with relatively modest salinity (0–2 ppt); this contrasts, for example, the work of Chambers *et al.* (2013) that compared 'fresh' (0.5 ppt), 'brackish' (13 ppt), and 'saline' (26 ppt) conditions. Future studies need to consider a broad range of exposure levels as ecosystem responses to salinity are highly nonlinear. Third, our study represented field conditions integrating prolonged exposure to varying salinity levels. Studies like Jackson & Vallaire (2009) assessed the response to short-term (55 days) increases in laboratory microcosms. In this context, our findings suggest that long-term shifts in C cycling may not be consistent with short-term disturbance-type responses, as hypothesized by Neubauer *et al.* (2013), and highlight a need for *in situ* work combined with manipulative studies.

Combined, these lines of reasoning help explain the strong correlation we observed between salinity and enzyme activity and provide several possible mechanisms by which salt water intrusion could directly influence decomposition rates. Salinity effects could also be indirect, and mediated through changes in microbial community structure. In this study, we observed a consistent shift in bacterial abundance and community composition with increased salinity (Fig. 4) and found these parameters to be correlated with the activity of several extracellular enzymes (Table 4). This could indicate that community structure (i.e., abundance and composition) regulates, at least in part, organic polymer breakdown and thus could constrain decomposition rates. Several prior studies have similarly documented a link between extracellular enzyme activity and changes in composition of the soil microbial community (e.g., Gallo *et al.*, 2004; Costa *et al.*, 2007; Morrissey *et al.*, 2013). The shifts in bacterial community structure we observed may be in part due to the physiological effects of increased saltwater, and numerous recent studies have demonstrated a link between salinity and microbial community composition (Bouvier & del Giorgio, 2002; Langenheder *et al.*, 2003; Langenheder & Ragnarsson, 2007; Berga *et al.*, 2012). Some researchers have even reported that salinity is the most important factor determining the distribution patterns of microorganisms across the globe (Lozupone & Knight, 2007; Auguet *et al.*, 2010). Besides ionic strength, saltwater intrusion brings with it specific ions that may affect microbial functional groups. For

instance, elevated sulfate can stimulate the growth of sulfate-reducing bacteria, which then outcompete the methanogens typically found in freshwater wetlands (Weston *et al*., 2006; Chambers *et al*. 2011; Weston *et al*., 2011).

In conclusion, salinity was found to be strongly related to bacterial community structure and decomposition activity in tidal wetlands ranging from fresh to oligohaline (Figs 3 and 4). The results of this study suggest that via abiotic and/or microbial processes, salinity is stimulating extracellular enzyme activity. This increased enzyme activity could reflect increased decomposition rates, contributing to the negative relationship between salinity and soil OM content in these ecosystems (Fig. 2). If saltwater intrusion does increase soil OM decomposition; previously freshwater wetlands may face reduced soil OM accumulation leading to lower accretion rates (Callaway *et al*., 1997; Craft, 2007). Consequently, these ecosystems may have more difficulty keeping pace with sea level rise than previously anticipated, potentially leading to ecosystem loss and large releases of stored C (DeLaune & White, 2011). More research on the mechanisms underlying salinity's regulation of enzyme activity and decomposition is sorely needed if we are to quantitatively predict salinity-induced changes in C cycling in tidal wetlands.

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Supporting Information

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

Table S1. Correlations coefficients (Pearson's *r*, top right half of the matrix) and *P*-values (lower left half) between all evaluated parameters. Enzyme (μmoles g-OM^{-1} h^{-1}) and bacterial abundance (*16S rRNA* gene copies g-OM^{-1}) data were natural log transformed (ln) prior to analysis. Bacterial community composition was represented using a Jaccard similarity matrix derived from *16S rRNA* T-RFLP; correlations presented below are from associated Mantel tests (r_M). Abbreviations are as follows: CBH, 1, 4- β-cellobiosidase; BG, β-1, 4-glucosidase; BX, β-D-xylosidase; AS, arylsulfatase; POX, phenol oxidase; AP, alkaline phosphatase; BGB, below-ground plant biomass; and AGB, above-ground plant biomass.