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Saltwater intrusion in context: soil factors regulate impacts of salinity on soil carbon cycling

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Abstract Salinization of freshwater ecosystems impacts carbon cycling, a particular concern for coastal wetlands, which are important agents of carbon sequestration. Previous experimental work using salt additions as a proxy for sea level rise, reveals widely divergent effects of salt on soil carbon processes. We performed a laboratory salt addition experiment on two different types of wetland soils (Ponzer muck and Hyde loam, both poorly drained organic soils) from the Coastal Plain of North Carolina. We used a commercial aquarium salt mix to make treatment solutions of 0, 2.5 and 10 ppt salinity and independently manipulated solution pH (5.5, 7.2, 8.8) for a full factorial experimental design. Our goal was to identify the effects of increasing ionic strength and increasing soil solution pH on soil carbon solubility and turnover. Microbial respiration and dissolved organic carbon solubility were depressed by marine salts, while pH manipulation alone had minimal effect. The addition of marine salts substantially reduced rates of carbon mineralization, reduced carbon solubility, and preferentially reduced the abundance of phenolic and aromatic organic molecules in solution. In the more acidic Ponzer muck, where salt additions dropped the pH from > 5 to < 4, we measured more substantial declines in DOC concentrations than in the base saturated Hyde loam. In contrast, in the base saturated Hyde loam, more marine salts remained in solution and the treatment effects on rates of carbon mineralization were more pronounced. Our results provide a clear demonstration of how ion exchange mechanisms result in indirect effects of salinization on the pH of soil solution and the solubility of organic matter. These indirect effects may explain much of the existing variation in reports of salt effects on soil carbon dynamics.

Keywords Coastal wetlands · Saltwater intrusion · Salinization · Carbon cycling · Soil carbon

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Introduction

The salinization of coastal wetlands due to sea level rise, storm surge and droughts is leading to forest mortality and substantial declines in biomass carbon stocks (Kirwan and Gedan 2019; Smart et al. 2020;

Ury et al. 2021). The consequences of soil salinization for below ground carbon cycling and sequestration are far less clear, with studies reporting both salt enhancement and salt inhibition of microbial activity in coastal wetland soils (Ardón et al. 2016, 2018; Helton et al. 2019; Doroski et al. 2019; Wen et al. 2019). To understand these divergent responses, we must consider the multiple pathways by which salt affects microbial activity and the effects of salinity on receiving soils. The addition of marine salts to soils does more than just increase the ionic strength of soil pore water; salt affects soil pH and base cation concentrations, and introduces new terminal electron acceptors (Tully et al. 2019). Additionally, the extent to which salinization alters soil carbon cycling is likely to be contingent on characteristics of the site itself (i.e., hydrology, soil properties), the level and duration of salt exposure, and prior exposure history.

There are multiple pathways by which salinity affects microbial activity in soil: directly by altering microbial activity, abundance, and community composition (Lozupone and Knight 2007; Luo et al. 2019; Dang et al. 2019; Rocca et al. 2020), and indirectly by affecting the quantity or quality of soil organic matter (Shainberg and Letey 1984; Jardine et al. 1989; Wichern et al. 2006; Mavi et al. 2012; Singh 2016). Increasing osmotic stress will lead to mortality for salt-sensitive microbes and major shifts in microbial community composition (Rocca et al. 2020). This may reduce rates of soil respiration or conversely, may stimulate the activity of salt-tolerant microbes through the release of labile organic matter following lysis of less tolerant microbes (Wichern et al. 2006; Singh 2016). Salinity affects the solubility, and therefore availability, of organic carbon within soil matrices (Mavi et al. 2012). Dissolved organic carbon (DOC) becomes less soluble with rising ionic strength due to flocculation (Shainberg and Letey 1984) and large organic molecules, such as phenolic compounds, may become less bioavailable through these processes (Ardón et al. 2016, 2018). This effect may however be offset by cation exchange resulting in the desorption of DOC from soil particles following salt addition (Jardine et al. 1989; Mavi et al. 2012). These shifts in DOC availability might interact with the direct effects of salinization on microbial community composition and activity.

The effects of salinization on soil carbon cycling are also contingent on pH, though little attention has been paid to pH in the literature on wetland salinization. Salinity and pH interact in two ways. First, the introduction of marine salts may result in a shift of soil pH due to cation exchange and alkalinization from base cations (Adams et al. 1984). Secondly, native soil properties including pH, and other related characteristics such as cation exchange capacity and base saturation, may interact with the effect of introduced salinity (Bache 2008). Independently, pH is an important control on soil microbial processes and the solubility of soil's constituents (Lauber et al. 2009) though pH is not always included as a parameter in ecosystem process models (Sulman et al. 2018). Given the importance of pH in moderating biochemical processes, we seek to investigate the interactions between salinity and pH in the context of wetland carbon cycling in the face of global change.

In this paper we conducted a laboratory soil salinization experiment in which we independently manipulated salinity and pH. Our pH-modified salt solutions were added to two different wetland soils collected from sites in coastal North Carolina. Both soils were obtained from within a 440-ha restored wetland, with nearly identical land use history, but vary in pH and base saturation. Our goal was to tease apart the effects of increasing ionic strength and altered pH on soil carbon stocks, soil solution DOC concentration and composition, and rates of microbial respiration; and to determine whether these effects were contingent upon pre-existing edaphic factors. We expected to observe antagonistic effects of increasing salinity and increasing pH on the solubility of DOC, but that salinity alone would reduce DOC and the concentration of high molecular weight phenolic and aromatic compounds. We predicted that rates of carbon mineralization would be suppressed by salinity but align with the treatment effects on DOC, with increasing pH possibly mitigating some of the stress caused by higher ionic strength. By investigating the effects of salinity on soil carbon availability and mineralization, we aimed to resolve inconsistent findings from prior literature surrounding the effect of salinity on carbon cycling in wetland soils.

Methods

Site description and sample collection

Soils were collected from the Timberlake Observatory for Wetland Restoration (TOWeR, see Ardón et al. (2010) for full site description) in Tyrrell County on the Albemarle-Pamlico Peninsula in Eastern North Carolina. The property was in agricultural production until 2004 when 440 hectares were restored to a 'preagricultural state' by way of extensive earth moving (ditch filling), rewetting, and planting 750,000 bare root saplings of native wetland trees. There are two major soil types at TOWeR, Ponzer muck and Hyde loam, which are the two predominant soil types of found across the entire Albemarle-Pamlico peninsula. For this experiment, we collected soils from a monitoring site within each soil type. Both soil types are characterized as very poorly drained, highly acidic, and rich in decomposing organic material. The Hyde series is a fine-silty, mixed, active, thermic Typic Umbraquults while the Ponzer series is a Loamy, mixed, dysic, thermic Terric Haplosaprists (Soil Survey Staff 2008, 2010).

There is a small elevation gradient across the property (less than 0.5 m) that drives hydrologic variation between the two sampling locations. The planting schema of the original site restoration also follows the topography such that each site contains a different mix of planted trees. The Hyde loam site has a slightly lower surface elevation, is regularly flooded, and thus is dominated predominantly by bald cypress (*Taxodium distichum*) as well as *Pinus taeda,* and *Salix nigra*. The Ponzer muck site has a slightly higher surface elevation, is less regularly inundated and so was planted mainly with species of oak (*Quercus nigra, Quercus phellos, Quercus falcate var pagodagfolia, and Quercus michauxii*). Natural recruitment of woody plants has been minimal at the Ponzer muck site, while an understory of *Acer rubrum* saplings and *Juncus effusus* is establishing at the Hyde Loam site.

Soils from both sites were collected on August 29th, 2020. On the day of sample collection, the sites were not submerged but at the wetter site (Hyde loam) the groundwater level was approximate at the surface of the ground and the soil was very saturated. After carefully removing the top layer of leaf litter, we used a small trowel to collect a sample from the top 5 cm of soil. Soil was collected from five randomly selected locations within a 10 × 10-m sample plot (established during a prior study). Samples were stored in sealed Ziploc bags, transported back to the lab on ice and stored overnight at 4 °C. The following day in the lab, equal portions from the five samples were composited into one larger sample for each site. Composited soils were sieved (2 mm) to remove roots and homogenize prior to experimental set up. A subsample from each site was reserved for analysis (in triplicate) of soil moisture and soil organic matter.

Experimental set-up and treatment application

The lab experiment was designed to assess the effect of salinity on wetland soil carbon with a particular focus on the interaction between salinity and soil pH. To accomplish this, we applied salt treatments to soils with highly divergent soil pH status (pH 4.0 versus 5.6, see Table 2) and measured soil carbon responses. We also manipulated the pH of the salinity treatments in a full factorial design to further disentangle the salinity by pH interaction. Treatment solutions (0, 2.5 and 10 ppt) were prepared with Instant Ocean® marine aquarium salt, a mixture that includes all of the major ions present in seawater, and deionized water (DI). Each solution was divided in three and aliquots were titrated with either dilute acid (HCl) or base (NaOH) until the desired pH was reached for a total of nine treatments (see Table 1). The pH treatment levels were chosen to match the resulting pH of each salt solution (i.e., solutions on the diagonal of Table 1 required no pH manipulation and contain only DI and Instant Ocean). The solution without salt (0 ppt) or pH manipulation (pH 5.5) is also referred to as the control treatment in our results. We acknowledge that the pH of our unaltered lab DI water is slightly lower than natural freshwater which is likely due to diffusion of atmospheric CO_2 forming carbonic acid. Our solution

Table 1 Experimental treatment salinity and pH in parentheses

	pH manipulation		
Salinity Treatment (ppt)	**0 (5.5)**	0 (7.2)	0 (8.8)
	2.5 (5.5)	**2.5 (7.2)**	2.5 (8.8)
	10 (5.5)	10 (7.2)	**10 (8.8)**

Bolding indicates treatments with no pHs manipulation

of pH 5.5 is therefore more akin to rainwater, than surface water, but is still consistent with what soils may experience in nature (USGS 2021).

Approximately 2.5 g of soil was weighed into filter funnels lined with Grade 1 Whatman filter paper and the exact weight was noted (see Fig. 1 for a schematic of the experimental setup). Treatments were randomly assigned, and soils were rinsed with treatment solutions in a 10:1 (volume: mass) ratio. Both the soil residue and the initial filtrate were used for further analysis. Filtrate solution was collected after approximately 25 min and the treated soils were allowed to air dry on the filter paper overnight to achieve samples that were relatively dry and thus reduce variability in soil moisture across samples. Half of the soil residue samples were transferred by mass into pre-weighed 40 mL amber glass I-Chem^{TM} vials for carbon mineralization analysis (54 total) over a 21-day incubation period at 20 °C. The remaining set of soil samples were transferred to falcon tubes for an identical incubation period followed by water extraction (54 total) in order to measure extractable DOC, phenolics and solution absorbance at a wavelength of 254 nm, $SUVA_{254}$, as a proxy for aromatic content.

Response measurements

Soil respiration assay vials were sealed with caps fitted with a PTFE-lined silicone septum to allow for headspace gas sampling via syringe. We measured headspace CO_2 concentrations with a LI-6250 gas analyzer (Li-core, Inc., Lincoln, Nebraska, USA) on days 1, 2, 3, 5, 7, 11, 14, 17, and 21. Vials remained sealed during the first 3 days of the incubation, then vented to prevent anoxia. Vials were vented and resealed 24 h prior to each subsequent measurement. The initial rate of potential C mineralization was calculated from the slope of the measurements over the first 3 days of the incubation and reported as μg C–CO_2 h^{-1} g C^{-1}, having been also corrected for the initial soil organic C content. The cumulative CO_2 collected over all measurements is also reported as the total μg C–CO_2 g C^{-1}. Following the 21-day incubation period, the vials were oven dried at 60 °C for 48 h

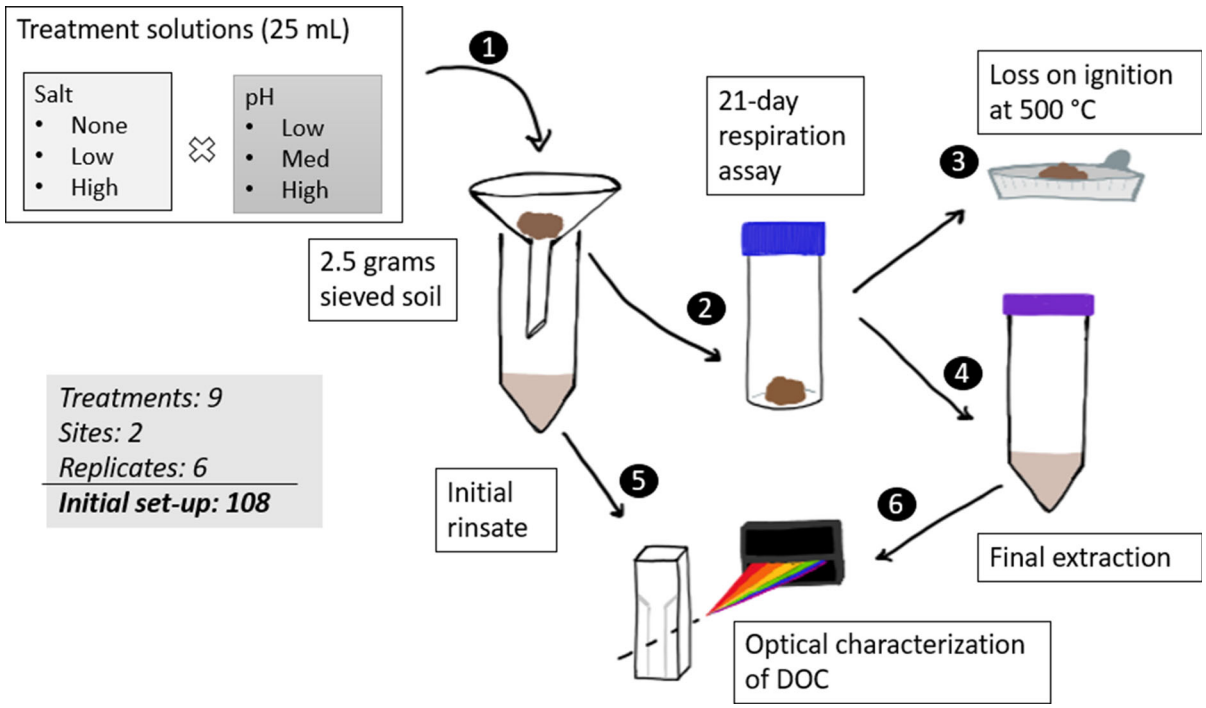

Fig. 1 Experimental setup began with (1) rinsing 2.5 g of field-moist soil with 25 mL of treatment solution. (2) The treated soil was transferred (analytically) to vials and sealed for incubation and respiration assay for 21 days. (3) Following the incubation, half of the samples were ashed in a muffle furnace to determine the remaining fraction of organic matter. (4) Remaining samples were extracted with 10:1 ratio of DI water. (5 and 6) Both the initial filtrate and final extract were characterized for optical properties of DOC (phenolic compounds and $SUVA_{254}$) and DOC was quantified using a TOC analyzer

to obtain a mass of dry soil for each assay and then heated in a muffle furnace at 500 °C for 4-h to obtain an estimate of the organic matter content remaining in each sample by loss on ignition.

The second set of incubated soils were kept in the dark, loosely covered for 21 days and then extracted with nano-pure water. A 10:1 water:soil (by mass) ratio was used to extract soils, first by shaking the slurry on an end-over-end shaker at 60 rpm for 4 h. Slurries were then allowed to settle over-night in the fridge and were then centrifuged at 3500 rpm for 15 min before being filtered through a 0.7 µm glass fiber filter. Both the initial filtrates and final extracts were analyzed for dissolved organic carbon (DOC), phenolic compounds, $SUVA_{254}$, pH, and conductivity. Water extractable dissolved organic carbon (DOC) was measured at the Duke River Center on a TOC-V combustion analyzer (Shimadzu Corporation, Kyoto, Japan). Solution pH and conductivity were measured with calibrated, hand-held probe devices (Hach H260G pH Meter, Loveland, CO; Oakton Con6 Acorn Series Conductivity Meter, Vernon Hills, IL). All measurements were conducted within 48 h of sample collection.

Phenolic compounds were measured using a colorimetric method (modified from Ohno and First (1998)). In a 24-well plate, 0.10 mL of Folin-Ciocalteu and 0.30 mL of 0.5 Molar $NaHCO_3$ was added to 1 mL of filtrate/extract, gently mixed and allowed to rest for color to develop for 4 h. UV–vis absorbance spectra were measured on a BioTek Epoch^{TM} 2 Microplate Spectrophotometer (Winooski, VT, USA) at 750 nm and calibrated against a standard curve made of vanillic acid (0–10 mg L^{-1}). The concentration of phenolic compounds was normalized by the DOC concentration and reported in mg phenolics mg DOC^{-1}. $SUVA_{254}$ was measured on the same spectrophotometer at 254 nm. Absorbance at 254 was normalized by the concentration of DOC in the sample as a characterization of the aromaticity of the carbon independent of the general level of organic matter and expressed as liters per mg DOC per meter (L mg DOC^{-1} m^{-1}) following Weishaar et al. (2003).

A second, separate set of soils collected previously from the same sites (July 2, 2020) were dried and sent to The North Carolina Division of Agriculture and Consumer Services (NCDA&CS) for routine soil testing of macro- and micro-nutrients (P, K, Ca, Mg, S, and Na; see https://www.ncagr.gov/agronomi/stmethod.htm for details) using Mehlich's standard methods (Mehlich et al. 1976; Mehlich 1984a, b).

Statistical analysis

All statistical analysis were conducted using R 4.0.1 (R Development Core Team 2020) with 'tidyverse' (Wickham et al. 2019) and 'ggplot' (Wickham 2016) packages. Due to relatively small sample sizes and that the majority of response variables are not normally distributed (Shapiro–Wilk's test), the Kruskal–Wallis test followed by a pairwise Wilcoxon Test was used to determine statistically significant ($p < 0.05$) differences between treatment groups within each site. The Scheirer–Ray–Hare extension of the Kruskal–Wallis test from the package 'rcompanion' (Mangiafico 2021) was used to test the significance of the interaction between treatment group and site.

Results

Site characteristics

Sites were selected due to their known difference in soil pH, however chemical analysis revealed further differences (Table 2) including a surprisingly large difference in base saturation. The Hyde loam had more than 4 × greater base saturation (79.5 ± 4.1%) than the Ponzer muck (17 ± 2.1%), with calcium and magnesium levels in the Hyde loam more than 7 × and 3 × greater than the Ponzer muck, respectively (Table 2). The cation exchange capacities of the two soils were similar, with the differences in base saturation perhaps due to differences in agricultural lime application or the hydrologic transport of lime during this site's prior history of agricultural use.

Treatment effects on soil salinity and soil pH

The experimental treatments significantly altered both the salinity and pH of the initial filtrate (Fig. 2) and the final soil water extracts [Supplementary Information (SI) Figs. S1–S3]. Our experimental pH treatments had far less of an effect on soil solution pH than our salinity treatment. Adding marine salts reduced the pH of the initial filtrate, relative to the control, by an average of 0.87 pH units ($p < 0.001$) for the 2.5 ppt treatment and by 0.65 ($p < 0.001$) for the 10 ppt

Table 2 Site characteristics and soil chemical properties

General site characteristics		
Soil series	Ponzer muck	Hyde loam
Dominant tree species	*Quercus spp.*	*Taxodium distichum*
Hydrology	Periodically inundated	Frequently inundated
Soil properties measured on experimental soil		
Soil moisture (%)	32.4 ± 0.1	36.7 ± 0.3
Soil organic matter (%)	11.2 ± 0.04	8.1 ± 0.1
Soil properties measured by NCDA&CS		
Humic matter (%)	2.9 ± 0.2	2.2 ± 0.2
pH	4.0 ± 0.05	5.6 ± 0.1
Base saturation (%)	17 ± 2.1	79.5 ± 4.1
Cation exchange capacity (Eq/g)	100 ± 18	99 ± 7.0
Na (Eq/g)	3.2 ± 1.0	1.7 ± 1.1
Phosphorus (μg/g)	99.8 ± 14	47.4 ± 3.9
Calcium (μg/g)	162 ± 50	1230 ± 160
K (μg/g)	112 ± 24	56.1 ± 5.1
Mg (mg/dm^3)	72.1 ± 14	177 ± 11
S (mg/dm^3)	60.0 ± 6.0	24.6 ± 2.6

Means plus or minus the standard deviation of five replicates

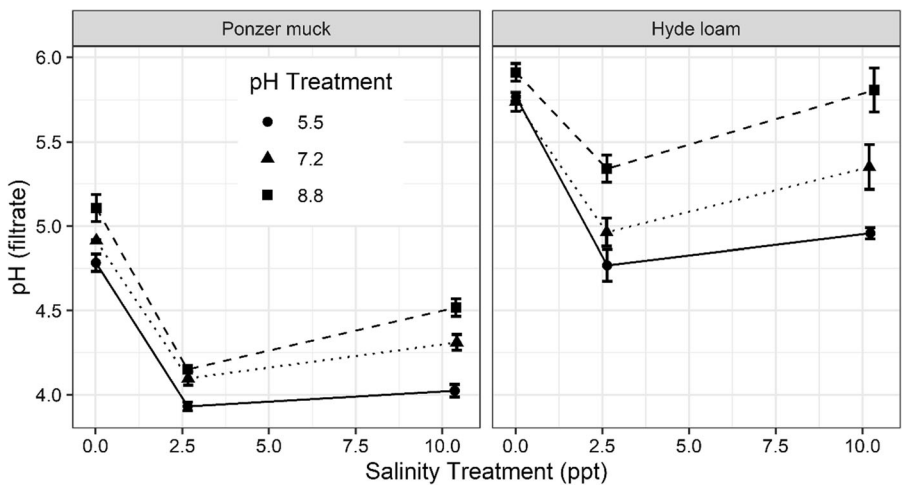

Fig. 2 The average measured salinity and pH of the initial filtrates with pH treatment groups indicated by symbol. Error bars indicate standard error for average pH measurements (standard error of measured salinity is < 0.05 and is not depicted here)

treatment in the Ponzer muck. In the Hyde loam the salinity treatment reduced the pH by an average of 0.78 pH units ($p < 0.001$) for the 2.5 ppt treatment and by 0.43 ($p < 0.001$) for the 10 ppt treatment. In both soils, our low salinity treatment had a larger effect on soil solution pH than our high salinity treatment.

There were no differences in pH of the final extract between pH treatment groups for either the Ponzer muck ($p = 0.44$) or the Hyde loam ($p = 0.35$) (complete test statistics for all results can be found in SI Table S1). The salinity treatments significantly raised the salinity of the final soil extracts by an average of 0.31 ppt ($p < 0.001$) for the 2.5 ppt treatment and 1.1 ppt ($p < 0.001$) for the 10 ppt treatment. This indicates the effective salinity experienced within the soil samples may have been significantly lower than target treatment levels, even when considering that some salt ions will bind to soil particles.

Our experimental manipulation of soil pH, in the absence of a salinity treatment had no measurable

effects on any response variables. Strong soil buffering clearly dampened the efficacy of our attempts to alter soil and soil solution pH. Given these findings, we focus the remainder of our results on the effect of salinity treatments on soil C response variables and the variation in these salinity effects across two soil types.

Salinity effects on soil carbon

Dissolved organic carbon in the filtrate of the initial treatment rinses was significantly ($p < 0.0001$) lower in the salt treatments as compared to the control by an average of 5.3 mg L^{-1} and 5.1 mg L^{-1} for the 2.5 ppt and 10 ppt salinity treatments, respectively (see SI Fig. S4). The difference between the 2.5 ppt and 10 ppt treatments was not significantly different ($p_{Ponzer} = 0.136$, $p_{Hyde} = 0.605$). The amount of carbon removed from soil samples in this first rinse accounts for approximately 0.1–0.2% of the total organic carbon content of the initial soils. Thus, although the salt treatments removed less C, we determine that the removal of organic material at this stage is negligible with respect to the remaining soil organic carbon (SOC) pool. This distinction is relevant for interpretation of subsequent experimental results.

There was a negative relationship between salinity and the initial (3-day) C mineralization rate ($p_{Ponzer} = 0.047$, $p_{Hyde} < 0.0001$) but the difference between groups was not statistically significant for the Ponzer muck (Fig. 3). The Hyde loam experienced a substantial reduction in C mineralization rate (47.0%) in the high salinity treatment compared to the control and there was a significant site by treatment interaction effect ($p = 0.0251$). The relationship between salinity and respiration in the Ponzer muck appears to follow the same general shape as the pH response (Fig. 2) whereas the relationship between salinity treatment level and C mineralization in the Hyde loam exhibits a more linear decline.

Total C mineralization (Fig. 4) over the 21-day duration of the experiment shows a similar pattern to the initial C mineralization rates (Fig. 3). There is a strong reduction in C mineralization over 21 days in the Hyde loam soil under the salinity treatments (24.1% and 43.2% for the 2.5 ppt and 10 ppt salinity treatments, respectively). We also measured a substantial reduction of C mineralization in the Ponzer muck with salinity (21.5% and 27.4% for the 2.5 ppt and 10 ppt salinity treatments, respectively), however the difference between the 2.5 ppt and 10 ppt treatment groups is not significant (Fig. 4). Unlike the initial rate, there is not a significant interaction between treatment and site on cumulative carbon mineralization over the entire 21-day incubation period (See SI Table S1 for full test statistics).

Adding marine salts significantly reduced water extractable DOC at the end of the incubation in both soil types (Fig. 5A). These treatment effects were more extreme in the more organic Ponzer muck soils, where we measured a 62.5% reduction of DOC in the 2.5 ppt treatment and a 77.8% reduction in the 10 ppt salinity treatment. The effects of salt addition on extractable DOC in the Hyde loam, were similar in direction but lower in magnitude, with a 33.9%

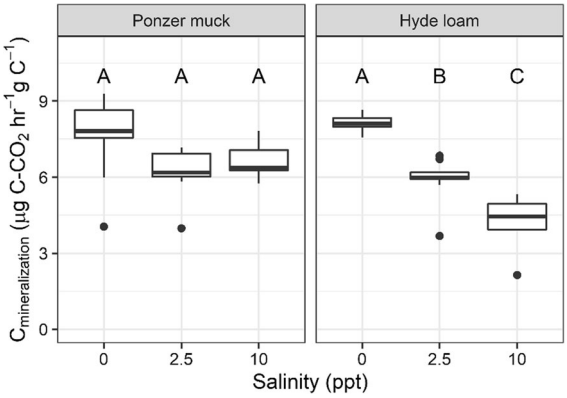

Fig. 3 Initial (rate over 3 days) C mineralization rate from the soil incubation assays on a per gram carbon basis. C mineralization rate per gram of dry soil is reported in SI Fig. S5. Different letters indicate a significant difference between treatment group within a site (Wilcoxon test, $p < 0.05$)

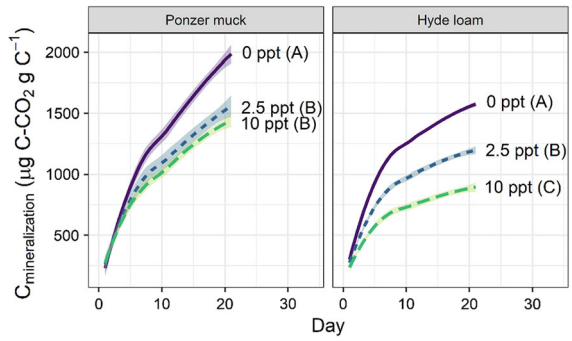

Fig. 4 Accumulation of CO_2 from mineralization assays over 21 days (Loess curve and standard error). Different letters in parentheses indicate a significant difference for total cumulative CO_2 production between treatment groups within a site (Wilcoxon test, $p < 0.05$)

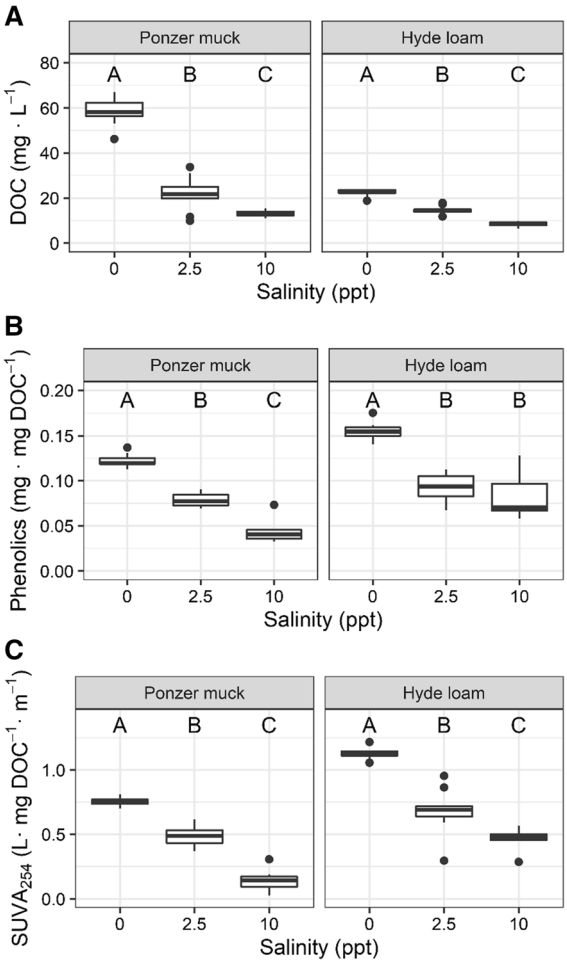

Fig. 5 Effect of salinity treatment on **A** DOC, **B** phenolic compounds and **C** $SUVA_{254}$. Different letters indicate a significant difference between treatment group within a site (Wilcoxon test, $p < 0.05$)

reduction of DOC in the 2.5 ppt salinity treatment and 62.0% in the 10 ppt treatment. Despite divergent responses between the two soils types, there was not a significant site by treatment interaction on the DOC response to salinity treatments ($p = 0.914$). We predicted that the more aromatic constituents of DOC would be particularly affected by salinity, and indeed measured significant declines in the total concentration of phenolic compounds (Fig. 5B) and $SUVA_{254}$ (Fig. 5C) and their relative contribution to total DOC in all salinity treatments (Fig. 5A, $p < 0.0001$). The effect of salinity on carbon quality is further demonstrated by decreasing the slope of the relationship between phenolic compounds and DOC (See SI Fig. S6). As we found for bulk DOC, these treatment effects were more extreme in the Ponzer muck than in the Hyde loam, but no significant interaction effect - was found between treatment and site salinity on either phenolics ($p = 0.0740$) or $SUVA_{254}$ ($p = 0.978$).

Finally, we examined the total organic matter content of soil samples following the 21-day incubation period. Salinity treatments resulted in greater retention of soil organic matter and all differences were statistically significant ($p < 0.01$) as shown in Fig. 6. The loss of organic matter was 11.6% (Ponzer muck) and 14.6% (Hyde loam) greater in the control treatment than the high salt treatment. In the control soils, the Hyde loam experienced a larger percent change from the initial soil organic content (− 15.7%) compared to the Ponzer muck (− 12.7%), however under elevated salinity, soil organic matter losses were minimal (between − 1% and − 4%) and comparable across soil types. Salt suppression of organic matter loss is assumed to be primarily through suppression of microbial respiration. We did not detect an overall site by treatment interaction effect on final SOM content ($p = 0.954$).

Discussion

We designed this experiment to look at the potential antagonistic effects of salinization and acidification on wetland carbon cycling. We found no evidence of antagonism, but instead that the indirect effects of our

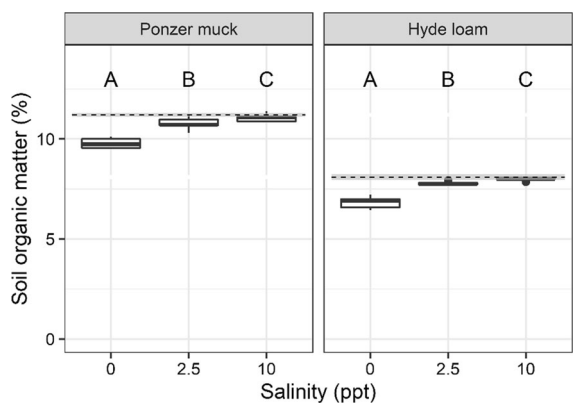

Fig. 6 Percent soil organic matter lost on ignition at 500 °C. The dashed line represents the initial, pre-treatment, soil organic matter content as measured by loss on ignition of a separate subsample of soil. Different letters indicate a significant difference between treatment group within a site (Wilcoxon test, $p < 0.05$)

salinity treatments on soil pH eclipsed the effect of the pH manipulation. We anticipated that seawater additions would increase soil solution alkalinity and that might offset the effects of the pH manipulations on carbon solubility. Instead, the acidification we observed following salt addition is likely to further reduce DOC solubility. The effective pH variation we achieved with our 5.5–8.8 pH treatment solutions led to variation of less than 0.3 pH units between treatment groups, and our experimental manipulation of pH had no effect on the majority of carbon response variables. Insight into the interaction between salinity and pH comes largely from the inherent differences between the two soil series. Examining the effects of salinity and pH in these different soils adds to our understanding of how salt impacts soil processes. Here we build on prior works that largely examine degree of salinity imposed on only one soil type (Ardón et al. 2016, 2018) and we raise new questions about the controlling effects of edaphic factors.

Our study demonstrates that the addition of marine salts displaces H^+ from cation exchange sites in these wetland soils (Fig. 2). The release of this exchangeable acidity lowers the pH of soil solution, further reducing organic matter solubility and compounding the effects of ionic stress on soil microbes with additional acid stress. The extent of this salt-induced acidification depends on the base saturation status of soils and on the ionic strength of the salinization event. Wetland soils with high base saturation status contain less exchangeable H^+ and are less prone to this indirect acidification effect. In base rich soils or under very strong salt treatments, more marine ions remain in solution as seen in studies by Kombo et al. (2005) and Weissman and Tully (2020). Base rich soils will thus be more resistant to salt-induced acidification but less resistant to salinization. In contrast, more acidic soils will react to salinization by sorbing marine ions and releasing H^+ into soil solution, making them less resistant to acidification but more resistant to salinization (Adams et al. 1984; Kissel et al. 2009; Minick et al. 2019). At sufficiently high levels of marine salt addition the acidification impact of releasing this exchangeable acidity is overwhelmed by sufficient quantities of marine base cations remaining in solution. Our experiment demonstrates that the soil chemical and microbial responses to salinization are dependent on initial soil conditions such as cation exchange capacity and base saturation. Importantly, the design of our experiment allows us to understand that the treatment strength of salt solutions does not translate to the effective salinity experienced within the experimental environment. Future work should attempt to account for this and distinguish between treatment target and treatment effect in their reporting.

Despite adding the same marine salt solution to both soils, the resultant effects of our salt treatments on soil solution pH, salinity and base cation concentrations differed. In our low salinity treatment, the addition of marine base cations displaced significant quantities of H^+ from the exchange complex, leading to a significant decline in pH in all treatment combinations (Fig. 2). This effect was far more pronounced in the Ponzer muck soil, where base saturation was only 17% and was more muted in the Hyde Loam soil, where base saturation was 80%. When we added our 10 ppt marine salt solution, the amount of added base cations was sufficient to neutralize the acidity of H^+ ion displacement. This means that the way microbes experience salinization is likely to depend on the extent of salt ion adsorption onto soil particles, the nature of ions exchanged or released from these complexes, and the ultimate composition of the resulting soil solution. The loamy soils of coastal wetlands have a high cation exchange capacity, and the extent to which these exchange sites are saturated with base cations will determine the acidification effects of low-level salinization. Widespread liming of agricultural fields in many coastal regions may lead to an accumulation of base cations in wetlands receiving agricultural runoff (Adams et al. 1984). Repeated exposure to marine salts (as observed in Ardón et al. (2017)) could also increase the base saturation status of exposed soils over time, thereby reducing the acidification impact of subsequent salinization events. The same features that allow a soil to resist acidification should also make them less able to dampen the osmotic stress impacts of salinization.

Adding marine salts to our soils reduced the solubility of organic carbon, altering both the quantity and quality of DOC leached from soil samples and, presumably, the form and availability of the soil carbon removed from solution. The reduction of DOC was more pronounced in the Ponzer muck soil, the more acidic soil, and is likely the effect of salt induced acidification of soil solution. Our findings are in agreement with previous work done on soils from this site in a study that demonstrated a similar reduction of

DOC export due to salinization and drought at the landscape scale (Ardón et al. 2016). The salinity treatments also impact the chemical makeup of DOC by reducing the solubility and in turn the absolute concentrations of phenolic compounds and $SUVA_{254}$ absorbing compounds (see SI Fig. S7) as well as their relative abundance within the total DOC pool (see SI Fig. S6). This suggests a potentially important change in the quality of soluble carbon following salinization, with large, aromatic compounds likely to be more susceptible to flocculation upon the addition of marine salts (Shainberg and Letey 1984). These changes to microbial carbon sources may have cascading impacts on soil carbon turnover and other biogeochemical processes (Creed et al. 2018).

Microbial respiration was significantly reduced by the addition of salt, although this effect was greater in the Hyde loam than in the Ponzer muck soil. We hypothesize that in this base saturated soil, more marine salts remain in solution and microorganisms experience greater osmotic stress following salt addition and thus we observed a more pronounced reduction of total respiration (Fig. 4). While salinity's direct effect on microbial activity and community composition is almost certainly at play (Lozupone and Knight 2007; Dang et al. 2019; Rocca et al. 2020), there may also exist an indirect effect following the influence of salt on organic carbon solubility. Specifically, flocculation and settling of DOC may limit its availability as a metabolite for microorganisms. The indirect effects of salinity on DOC as a control for microbial respiration are not often discussed in the literature (Neubauer 2013), but provide a case for a more holistic approach to investigating soil carbon dynamics. More work in this area is necessary to tease apart the contribution of the indirect effects of salinity on DOC versus the direct effects of salinity stress on soil microbes.

Our demonstration that marine salinization suppressed microbial activity in these freshwater wetland soils is consistent with several prior experiments (Ardón et al. 2016, 2018; Helton et al. 2019; Doroski et al. 2019; Wen et al. 2019), though numerous studies have observed the opposite trend (Weston et al. 2006, 2011; Chambers et al. 2011; Neubauer 2013). Mechanisms supporting enhanced respiration following salt exposure include the fertilization effect of low dose salt treatments and mobilization of nutrients and labile carbon following cell death (Wichern et al. 2006; Herbert et al. 2015). Explanation for these discrepancies within the literature may include geographic differences, experimental duration, treatment strength, and other experimental design conditions. We hypothesize that edaphic factors, including soil pH, may be an important, and often overlooked, explanatory variable.

Our findings support our initial predictions for carbon responses to salinity treatments alone—the salinity treatments reduced microbial respiration, the solubility of organic carbon, and the aromaticity of DOC. The compounding effects of soil salinization, salinization induced acidification and the reduced DOC solubility that results may help explain why we measured such dramatic impacts of our salinity treatments on total soil carbon stocks. At the conclusion of the 21-day incubation period, while the control soils experienced a − 12.7% (Ponzer muck) and − 15.7% (Hyde loam) change in organic matter content (compared to pre-experimental soils), the highest salt treatment soils experienced only about − 1% change in organic matter content (both soil types). This lab-based experiment provides clear evidence that site conditions and baseline soil properties will mediate the impacts of salinization in soil carbon cycling. Future experimental work on this subject should attempt to document baseline soil conditions and treatment effects on both salinity and soil pH as well as consider the impacts of duration of exposure to salinity and varying hydrologic conditions, so that we may adequately compare between sites and synthesize a more complete understanding of coastal carbon dynamics in the context of sea level rise.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data availability The datasets generated and analyzed here are available in the FigShare data repository: https://doi.org/10.6084/m9.figshare.14489094.

Code availability All code for data analysis is available at https://github.com/EmilyUry/Paper-Salt-pH-Experiment and can be run in R, an open-source statistical programming environment.

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