The Biogeochemical Consequences of Saltwater Intrusion to Freshwater Wetland Sediment

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Abstract

Saltwater intrusion driven by water extraction, coastal modifications, and climate change may alter the biogeochemical cycling of freshwater coastal wetlands. Anaerobic respiration in freshwater wetlands is typically dominated by methanogenesis, leading to a high methane (CH^4) flux, but the availability of sulfate (SO_4^{2-}) in seawater may shift the dominant pathway to SO_4^{2-} reduction, decreasing CH^4 flux. Seawater may also impact nitrogen cycling by releasing ammonium (NH_4^+) from soil and causing salinity and hydrogen sulfide stress to nitrifiers and denitrifiers. This experiment tests the soil biogeochemical impacts of artificial seawater amendments to intact sediment cores taken from a freshwater coastal wetland on the Albemarle Peninsula of North Carolina. Intact cores were assigned to one of four experimental treatments designed to compare the impact of surface and subsurface saltwater exposure on sediment biogeochemistry. Cores received surface water treatments 2-3 times per week and were exposed continuously to subsurface treatments. Gas flux and porewater were sampled 9 times over the 20-week experiment. Saltwater added to the soil surface raised soil solution to 10.4 ppt average salinity by the end of the experiment and led to significant increases in NH_4^+ concentration and significant declines in dissolved organic carbon (DOC). In surface saltwater exposed cores we measured a significant increase in nitrous oxide (N_2O) production but no significant change in carbon dioxide (CO_2) or CH_4 flux. Surface exposure treatments led to significant reductions in microbial biomass, and all salinity treatments (regardless of direction of exposure) had significant reductions in carbon mineralization and respiration efficiency by the end of the experiment. Our results suggest that saltwater exposure altered microbial biomass and function, and that surface water salinization will have more immediate and measurable impacts on biogeochemical cycling in these soils than exposure to saline ground water.

Contents

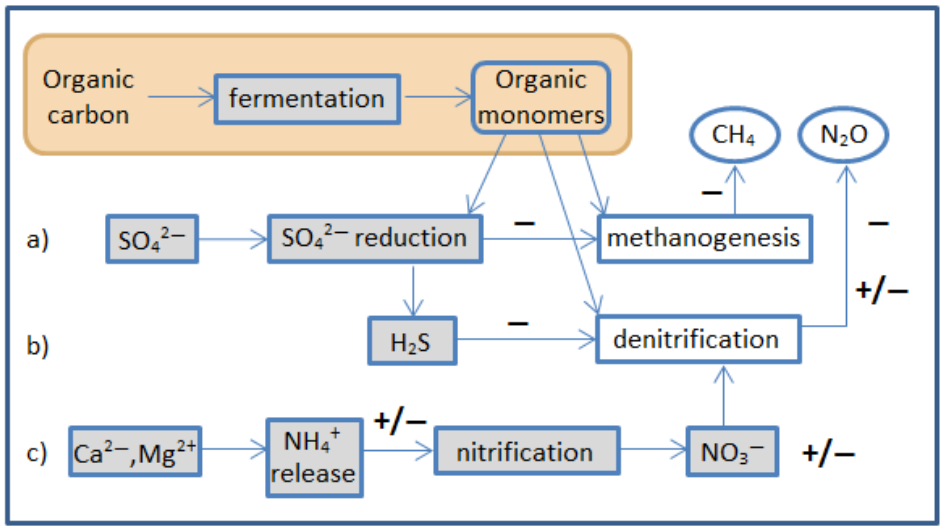
Introduction

As a consequence of gradual sea level rise, soil oxidation and sustained seasonal droughts, coastal plain ecosystems are experiencing increasingly frequent and long-lasting saltwater intrusion, the landward movement of saltwater into historically freshwater habitats. The boundary between fresh and saltwater typically depends on hydrogeologic characteristics, including surface and subsurface freshwater flow, hydraulic conductivity of aquifer sediments, and history of local sea level fluctuations. Coastal groundwater extraction is historically the most prevalent cause of saltwater intrusion (Barlow 2003), reducing freshwater influence and allowing denser saltwater to penetrate inland, though in some areas such as South Florida wetland drainage to lower the water table for agriculture is a major cause (Barlow and Reichard 2010). Climate change impacts will likely exacerbate the problem, with sea level rise and intensified storm driving saltwater inland, and higher frequency and severity of droughts lowering freshwater influence (Sherif and Singh 1999, Steyer et al 2007).

The hydrogeologic setting determines whether saltwater intrudes through surface or subsurface flowpaths (Barlow and Reichard 2010). Coastal wetlands typically receive most water input from surface flow, because upward movement from the subsurface is limited by clay and organic matter deposits, though groundwater may seep up through more permeable areas with thinner organic matter. In some cases where the underlying aquifer is sand-based, tidal creeks accumulate coarser sediments even as surrounding wetland accumulates organic matter, maintaining a conduit of flow with the subsurface (Howes et al 1996). Since groundwater extraction has primarily led to groundwater intrusion (Barlow and Reichard 2010), it will primarily affect lower wetland sediment layers, assuming the saltwater seeps upward from the aquifer, while droughts and sea level rise likely cause both modes of intrusion, also affecting surface sediments.

Saltwater intrusion has been shown to stress salt-intolerant wetland vegetation (McKee 1989, Howard and Raffery 2006) and displace freshwater macroinvertebrates and other fauna (Kang and King 2012), yet the impacts on biogeochemical cycling in wetlands are unclear. Saltwater intrusion exposes freshwater sediment to a new suite of chemicals and nutrients, delivering sulfate (SO_4^{2-}) and base cations (Figure 1). SO_4^{2-} is an important electron acceptor for anaerobic microbes, and the provision of SO_4^{2-} is expected to suppress methanogenesis through replacement with more efficient SO_4^{2-} reduction, also enhancing organic matter mineralization (Weston et al 2006, Mishra et al 2003). SO_4^{2-} reduction produces hydrogen sulfide (H_2S), which is toxic to some nitrifiers and denitrifiers, potentially reducing denitrification and fluxes of nitrous oxide (N_2O) (Rysgaaard et al 1999, Caffey et al 2003). The provision of base cations from seawater (K^+, Ca^{2+}, and Mg^{2+}) can displace ammonium (NH_4^+) bound to soil particles (Seitzinger 1991), and while some studies suggest this reaction ultimately decreases nitrification and denitrification by exporting NH_4^+ (Gardner et al 1991, Seitzinger 1987), the short term implications of NH_4^+ mobilization are unclear.

Figure 1. Expected major impacts of saltwater ions (SO_4^{2-}, Ca^{2-}, Mg^{2+}) on microbial use of limiting organic monomers: a) SO_4^{2-} reduction replaces methanogenesis, decreasing CH_4 flux, b) H_2S toxicity to denitrifiers decreases N_2O flux, and c) Ca^{2-} and Mg^{2+} displace bound NH_4^+ from soil, with unclear implications for nitrogen availability and N_2O flux. Baseline conditions in white.



In this study, I tested the biogeochemical impacts of seawater amendments to intact sediment cores from a freshwater coastal wetland of the Pamlimarle Peninsula in coastal North Carolina. I attempt to differentiate between surface water and groundwater impacts by having some cores placed in saltwater, others receiving saltwater input from the top, and some receiving saltwater through both modes. Chloride (Cl^-) and SO_4^{2+} concentrations, trace gas fluxes and dissolved gases (CO_2, CH_4, N_2O), dissolved organic carbon (DOC), nitrogen content (NH_4^+, NO_3^-), and phosphate (PO_4^{3-}) were measured throughout the 20 weeks of the experiment, and reduced iron concentration was measured at the experiment conclusion. Microbial biomass at the beginning and end of the experiment were approximated by measuring substrate induced respiration (SIR). Carbon mineralization and CH_4 potential, which approximate labile carbon content and methanogen biomass, were measured at the end.

Methods

Experimental Set-up

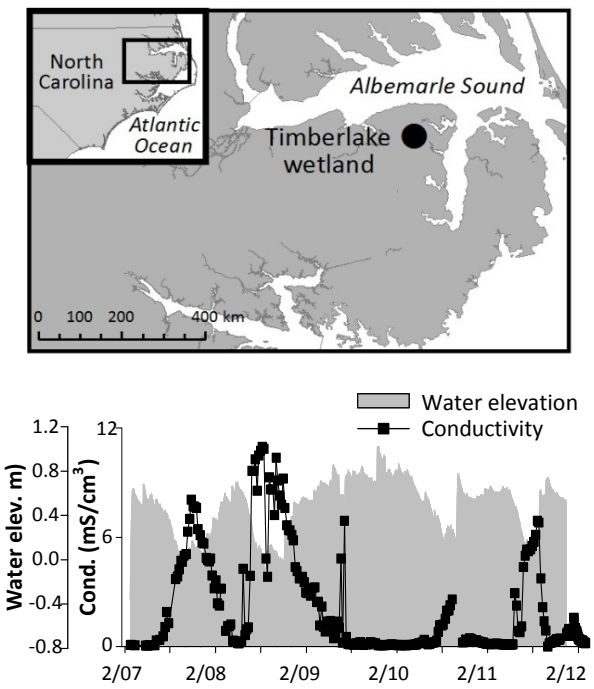
Source of soil cores

The cores were collected from the 440-ha Timberlake Restoration Project wetland in the Albemarle Peninsula in Tyrell County, within the North Carolina coastal plain (Figure 2). Much of the peninsula was historically pocosin wetland, though nearly three-quarters of the area was deforested and drained for agriculture by the 1980s (Richardson 1983; Carter 1975). The Timberlake wetland is a former agricultural

field that underwent restoration activities from 2004 to 2007, restoring hydrology and wetland vegetation (Ardon et al 2010). Although Timberlake and the surrounding areas are formally classified as non-tidal wetlands, owing to the Albemarle Sound's narrow astronomical tidal range, they are subject to the wind tides of the Sound (Poulter et al, 2008, Titus and Strange 2008).

Beginning in 2007, conductivity monitoring of the Timberlake wetland has revealed several episodes of saltwater intrusion coinciding with late summer droughts (Figure 2). With fresh water levels low, wind tides can drive saltwater from the Albemarle Sound further inland. Salinity patterns vary spatially within Timberlake, and the source of the cores is from an area without documented intrusion.

Figure 2. Albemarle Peninsula and conductivity record (2007-2012) at Timberlake



Experimental design

We collected 34 cores from a unvegetated saturated area with 0 – 5 cm of water in May, using 30-cm, 5-cm diameter, core sleeves. We brushed aside dead plant matter from the soil surface before taking each core. Sediment was cored to a depth of 25 – 27 cm. Cores were sealed on the top and bottom and stored in coolers for transport. Five cores were analyzed for initial soil characterization, separating each core into 5-cm increments; any sediment beyond a depth of 25 cm was included in the 20-25 increment. Of the remaining 29 cores, 15 cores with less than 3 cm headspace were extruded and sediment was cut off the bottom, creating 3 centimeters headspace to hold surface water additions. We drilled holes in the cores 10 cm below the sediment surface and installed lysimeters for porewater sampling. Core bottoms were fit with a superfine filter, to allow water to pass through. Five Rubbermaid© containers were set up with 10 cm of artificial freshwater and the cores were placed inside, and the lid affixed, with holes for the cores to emerge through. We began the surface watering regime of adding 25 – 50 mL of artificial freshwater (Table 1) to the tops of cores 3 times per week, and allowed the cores to equilibrate with only freshwater exposure for 4 weeks.

Table 1. Contents of artificial freshwater and seawater (µmol/L)

	Freshwater	Saltwater
Salinity (ppt)	5.05	0.08
Cl^-	79,365	227
Na^{2+}	58,867	875
Mg^{2+}	8,154	0.10
Ca^{2+}	1,601	0.30
K^+	7,888	88.2
SO_4^{2-}	3,289	34.0
HCO_3^-	332	660
$H_3BO_3^-$	64.69	
Sr^{2+}	13.03	
Br^-	12.00	
F^-	1.02	

After 4 weeks, to begin the baseline period, we sparged the standing water with N2 for 15 minutes to create anoxic groundwater, continuing until dissolved oxygen levels were below 0.5 mg/L, and we added a layer of mineral oil 5 mm thick. Through the course of the experiment, we bubbled N_2 for 10 minutes roughly every other week to maintain low oxygen. During the pre-treatment period, we measured anion (NO_3^-, SO_4^{2-}, Cl^-) concentrations in porewater three times and eliminated 4 cores because of unusually high concentrations of one or more anions relative to the entire set. After the pre-treatment period, we harvested 5 cores for time zero soil characterization, separating each core into 5-cm increments.

We rearranged the 20 remaining cores into four treatments, evenly distributing baseline anion concentrations between groups, and evenly distributing cores that were extruded to 3 cm headspace, since extrusion may have caused some impaction. The four treatments were a control group, receiving 25 – 50 mL freshwater to the tops of cores 3 times per week while sitting in fresh groundwater; a surface exposed group representing surface saltwater intrusion, receiving saltwater in the tops of cores while sitting in fresh groundwater; a subsurface exposed group representing groundwater intrusion, receiving freshwater in the tops of cores while sitting in saltwater; and a surface and subsurface exposed group representing intrusion through both modes, receiving saltwater in the tops of cores and sitting in saltwater. The experiment was maintained for 20 weeks. Cores were broken down and the top 0-5 cm and 5-10 cm of soil were analyzed.

Table 2. Sampling schedule

Experiment timeline	• Equilibration: 4 weeks (5/15–6/10) • Pre-treatment measurements (baseline): 2 weeks (6/11 – 6/20) • Time zero soil analysis (6/20) • Experimental treatments: 20 weeks (6/26 – 11/13), with 6/26 as Week 0 (time zero)		
Type	**Measurement**	**Frequency**	**# Sample Events**
Gas flux	CO_2, CH_4, N_2O	1 baseline measurement, Weeks 0, 1, 2, 3, 4, 10, 14, 20	9
Dissolved gas in porewater	CO_2, CH_4, N_2O	Weeks 1, 2, 3, 4, 10, 14, 20	7
Porewater chemistry	Anions (Cl^-, SO_4^{2-}, NO_3^-)	1 baseline measurement, Weeks 0, 1, 2, 3, 4, 10, 14, 20	9
	DOC, NH_4^+, PO_4^{3-}	Weeks 0, 1, 2, 3, 4, 10, 14, 20	8
	Reduced iron (FeII)	Week 20	1
Microbial response	Substrate induced respiration (SIR)	Initial, time zero, end (Week 20)	3
	Methane potential, carbon mineralization	End (Week 20)	1

Gas sampling

We sampled surface gas flux of CO_2, CH_4, and N_2O, measuring once a week during the baseline and first four weeks, and every 4 – 6 weeks during the remaining 4 months. Gas sampling occurred roughly 3 hours after watering. Cores were fitted with a gas-tight rubber cap, and a time zero sample to measure background concentration was immediately taken by injecting 10 mL CO_2-free air into the headspace through the cap's septa and extracting 10 mL of gas sample and injecting it into evacuated 9-mL gas vials. After 120 to 150 minutes, a second set of samples was taken. Headspace volume was estimated by measuring depth from the core top to the soil surface, or to the water surface if standing water was present. Gas concentrations were measured within one week on a Shimadzu 17A gas chromatograph with electron capture and flame ionization detectors (Shimadzu, Kyoto, Japan). Gas production ($g/m^2/day$) was derived using the concentration difference and time elapsed between time zero sampling and the second sampling, and the headspace volume.

We measured CO_2, CH_4, and N_2O dissolved in the porewater, collecting porewater immediately after gas sampling. A needle was attached to each lysimeter and an evacuated 9-mL gas vial was stuck onto the needle. Vials were removed after several minutes or after they'd collected 2-5 mL porewater. Generally only 2 – 3 cores per treatment gave at least 2 mL after 20 minutes, after which point the vials were detached. For each sample, we injected N2 to over-pressurize the vial 3 – 4 mL. Vials were shaken for 15 seconds to equilibrate gases between liquid and headspace, then a headspace gas sample was taken and added to a final evacuated vial. N2 was added to the final vial to bring the total volume to 10 mL. Headspace gas concentrations were analyzed on the gas chromatograph within one week. Dissolved gas concentrations were calculated using headspace gas concentrations, the volume of porewater collected, and Henry's Law constants.

Cores varied in the rate that surface water percolated through: though all cores had standing water at the beginning of gas flux sampling, some were dry by sampling conclusion, potentially causing oxic conditions in the top soil layers, which would affect gas flux. Most weeks, it was noted which cores had standing water or appeared fully saturated at the surface. Percolation rate was also assessed midway through the experiment by adding 100 mL water and measuring how far the water level dropped in 2 hours, with rates ranging between 0.2 to 7.8 mm/hr, Control cores percolation rates were significantly lower than other cores, and SW-top rates were higher (Table 3).

Porewater chemistry sampling

Porewater was collected for anions (Cl, SO_4^{2-}, NO_3^-), dissolved organic carbon (DOC), NH_4^+ and PO_4^{3-}. Sampling occurred once a week during the baseline and first four weeks, and every 4 – 6 weeks during the remaining 4 months. Lysimeters were affixed with a 10-mL plastic syringe held in the open position to create a vacuum and extract after 12 hours, water was collected into 10-mL plastic vials and frozen until analysis. Before analysis, samples were thawed and brought to room temperature.

Samples were run for anion content on an ICS-2000 Ion Chromatography System with an AS4A anion column and KOH effluent generator (Dionex, Sunnyvale, CA, USA). DOC and TN were measured on a total organic carbon analyzer with a total nitrogen module (Shimadzu, Kyoto, Japan). NH_4^+ and PO_4^{3-} was

measured run on a Lachat QuikChem 8500 autoanalyzer (Lachat Instruments, Loveland, CO, USA) using method 10-107-06-1-J for NH_4^+ and method 10-115-01-1-A for PO_4^{3-}.

Reduced iron was measured at the last timestep by extracting 1 mL of porewater with the syringe and immediately adding it to 1 mL of a ferrozine reagent. Samples were run on a LKB Biochrom Ultraspec II spectrophotometer (Biochrom, Holliston, MA, USA).

Microbial Response

Microbial biomass was estimated using substrate induced respiration (SIR) in the initial soil assessment, time zero, and at the end for the 20 experimental cores. For the initial and time zero assessments, cores were divided into five 5-cm depth ranges; for the end assessment, only the top 0-5 and 5-10 cm ranges were analyzed. The SIR protocol was modified from West and Sparling (1986). Four grams wet soil and 10 mL of a yeast and water solution were added to each sample, delivering 20 mg yeast per g dry soil, and samples were capped and shaken on a rotary shaker table at 150 rpm for 15 minutes. Time zero headspace CO_2 concentrations were measured by injecting 1 mL CO_2-free air into each vial and extracting 1 mL of sample. The vials were returned to the shaker table, and headspace CO_2 concentrations were measured again after 2 and 4 hours. Samples were run immediately after extraction on a LI-6200 Portable Photosynthesis System and LI-6250 CO_2 analyzer (Li-cor, inc., Lincoln, Nebraska, USA). The average respiration rate (mg CO_2-C/d/g dry soil) over the 4-hour period was calculated.

Aerobic carbon mineralization and anaerobic CH_4 potential were measured at the end for the 20 experimental cores for 0-5 cm and 5-10 cm depth. For carbon mineralization, 1 g wet soil and 2 mL AFW were added to a serum vial. An initial gas sample was collected by adding 1 mL CO_2-free air to the vial and extracting a 1-mL gas sample, and adding it to a 9-mL gas vial with 9 mL of N_2. Sampling was repeated after 3 and 7 days. For CH_4 potential, 5 g wet soil and 10 mL AFW were added to a serum vial, and vials were evacuated and filled with N_2 to an even pressure. An initial gas sample was collected by adding 1 mL N_2 to the vial and extracting a 1-mL gas sample, and adding it to a 9-mL gas vial with 9 mL N_2. Sampling was repeated after 3 and 7 days. Gas samples were analyzed on the gas chromatograph within one week of collection.

Respiration efficiency, a measure of carbon quality, was evaluated by dividing carbon mineralization by SIR. This yields a ratio that shows the amount of CO_2 respired using available carbon in the soil, compared to CO_2 respired when microbes are supplied with substrate.

Statistical Analysis

All data was analyzed using R v. 2.13.1. On-going measurements (gas flux, dissolved gas, and concentrations of Cl, SO_4^{2-}, nitrogen, and DOC) were analyzed using a one-way repeated measures ANOVA. The main result considered is the F-statistic and p-value for the interaction of treatment and sample event, which reveals a significant treatment effect that varies over time. The F-statistic and p-value for treatment alone show whether any treatment was steadily different, and for sample event alone, they show whether the cores exhibited a similar pattern over time regardless of treatment.

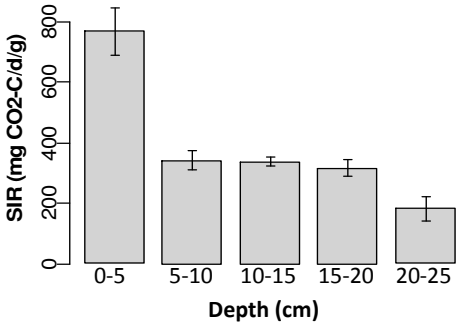
Measurements only taken at fixed time points (reduced iron concentrations, soil characteristics, and microbial parameters) as well as estimated total gas flux over the course of the experiment were analyzed using a one-way ANOVA, and variables with significant ANOVA results were analyzed using a multiple comparison ANOVA. The impacts of certain characteristics, such as percolation rates and whether cores were wet or dry by the end of gas flux sampling, on gas fluxes were analyzed separately using linear regression analysis.

Results

Initial soil characteristics

Initial and time zero dry mass ratios were similar and did not vary consistently by depth, ranging from 0.30 to 0.66. Ash-free dry mass ratios at time zero ranged from 0.49 to 0.81 and also did not vary by depth.

Figure 3. Pre-treatment SIR by depth



SIR at time zero decreased by depth, ranging from 614.6 – 988.8 mg CO_2-C/d/g dry soil in 0-5 cm depth, 282.1 – 425.6 in 5-10 cm, 292.7 – 376.4 in 10-15 cm, 255.0 – 384.0 in 15-20 cm, and 102.2 – 321.2 in 20-25 cm (Figure 3). SIR in initial cores was considerably lower than for the time 0 measurements. I consider the time 0 analyses less representative of pre-treatment conditions, because initial cores were analyzed shortly after removal from refrigeration. Rates in the top two layers (0-10 cm) ranged from 84.6 to 494.5, and the lower three layers ranged from 4.1 to 121.6.

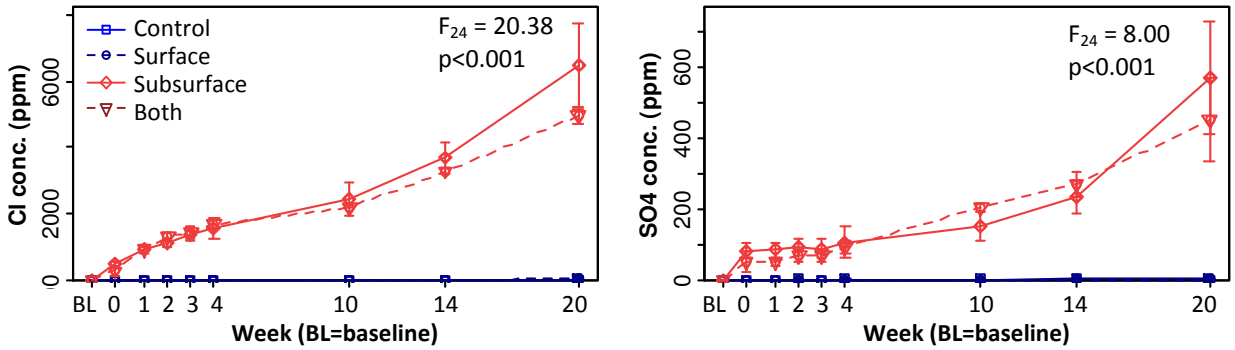
Treatment description

Cl^- concentrations ranged from 13 to 37 ppm pre-treatment, increasing significantly over time in surface exposed cores and surface + subsurface exposed cores to a range of 3,403 to 10,408 ppm in 10-cm depth lysimeter samples by week 20 (Figure 4). Assuming the ratio of total salinity to Cl^- remained the same as in the artificial saltwater (1.796), this represents a salinity range of 6.1 to 18.7 ppt. Most control and subsurface exposed cores remained within the pre-treatment range for the experiment duration, except two subsurface exposed cores were 102 and 148 ppm by week 20. Across both surface exposed treatments, average Cl^- had already increased in week 0 to 395 ppm, indicating surface applied sea salts had reached lysimeters within 6 hours of the first salinity amendment.

SO_4^{2+} concentrations followed similar trends, ranging from 0.13 to 1.90 ppm in pre-treatment, increasing significantly over time in both surface exposed treatments to a range of 144.7 to 1065.9 ppm by week 20 (Figure 4). Average SO_4^{2+} in control and subsurface exposed cores increased slightly to 7.8 and 2.6 ppm by week 20. Similar to Cl^-, average SO_4^{2+} across both surface exposed treatments had already increased in week 0 to 70.5 ppm.

Cl^- and SO_4^{2+} both show similar patterns between surface exposed and surface + subsurface exposed cores, and between control and subsurface exposed cores. These similarities suggest that saltwater had not diffused upward to the lysimeter levels in either subsurface exposed treatment, except for the two subsurface exposed cores which rose slightly.

Figure 4. Chloride (Cl^-) and sulfate (SO_4^{2-}), with repeated measures ANOVA F-statistic and p-value



Solute Treatment Responses

NH_4^+ concentration

NH_4^+ concentrations ranged between 0.23 and 31.17 ppm, remaining low in control and subsurface exposed cores over time and significantly increasing in both surface exposed groups. At week 0, average NH_4^+ across control and subsurface exposed cores was 1.75 ppm and both surface exposed treatments averaged 3.96. The difference in NH_4^+ in week 0 ($F_3 = 3.23$, $p=0.052$) suggests a response within the 6 hours after the first saltwater amendment and the porewater collection. NH_4^+ in both surface exposed groups increase again in week 1, remain steady through week 10, and again increase in weeks 14 and 20.

Though the repeated measures ANOVA gave non-significant results for the treatment effect over time, the effect of treatment alone is significant ($F_3 = 20.22$, $p<0.001$), indicating that both surface exposed treatments were consistently higher than control and subsurface exposed cores. The inclusion of pre-treatment values would have likely shown a similar baseline across treatments, leading to a more significant treatment effect over time. A re-run of the ANOVA setting all baseline values to the average week 1 NH_4^+ concentration of control cores (1.56 ppm) yields significant results ($F_{24} = 2.01$, $p=0.008$).

Dissolved Organic Carbon

DOC concentrations ranged between 8.7 and 207.6 mg/L, remaining fairly steady in control and subsurface exposed cores and declining significantly in both surface exposed treatments (Figure 5). At week 0, average DOC across control and subsurface exposed cores was 142.1 mg/L and both surface exposed treatments averaged 68.8. Similar to NH_4^+, the difference in DOC values in week 0 ($F_3 = 7.75$, $p=0.002$) suggests a response within the 6 hours after the first saltwater amendment and the porewater collection. After dropping further by week 1, DOC in both surface exposed treatments stabilized and remained between 8.7 to 44.7 mg/L. Subsurface exposed cores began to drop after week 10, each declining 35-77% by week 20.

NO_3^- concentration

NO_3^- concentration ranged from below detection limit to 7.1 ppm and showed no significant treatment effect over time (Figure 5). Concentrations were typically on the low end of this range, with 69% of readings below 0.5 ppm. While average rates were typically higher in the surface exposed treatments, this difference existed in the pre-treatment period. Though treatments are not significantly different, NO_3^- in control and sub-surface exposed cores stays below 0.5 ppm, while rates across cores in both surface exposed treatments are more variable, with 4 out of 10 cores regularly exceeding 1.0 ppm.

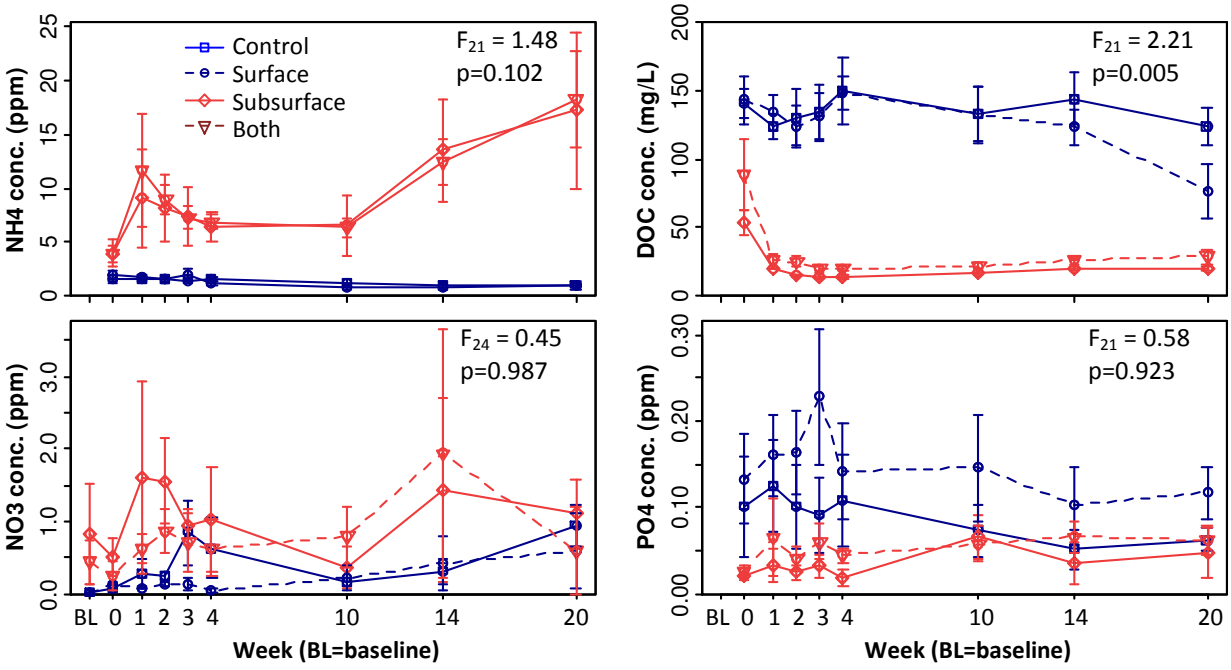
PO_4 concentration

Phosphate (PO_4) concentrations ranged from 0.005 to 0.474 ppm and showed no significant treatment effects over time (Figure 5).

Reduced iron concentration

Reduced iron values, measured only in week 20, ranged from 0.9 to 24.5 ppm and were significantly higher in both surface exposed saltwater cores than in control and subsurface exposed cores (Table 3). Both surface exposed treatments together averaged 15.9 ppm while control and subsurface cores averaged 3.7 ppm.

Figure 5. Porewater solute concentrations, with repeated measures ANOVA F-statistic and p-value



Trace Gas Response

CH_4 flux and dissolved content

CH_4 flux ranged from below detection to 71.6 g/m^2/day and showed no significant treatment effects over time (Figure 6) or in estimated cumulative CH_4 flux throughout the duration of the experiment, which ranged from 1.6 to 7,713 mg (average of 1,797 mg) over 4 months for individual cores (Table 3).

Flux values were skewed towards 0, with 74% of values below 0.1 g/m^2/day. CH_4 flux in cores from both surface exposed treatments was consistently low, together only exceeding 1 g/m^2/day for 5% of readings, while control and subsurface cores exposed exceeded 1 g/m^2/day for 26% of readings. The high average flux in week 10 for the surface exposed treatment results from a single core with a flux of 62.3 g/m^2 /day, and removing this core drops the average from 12.8 to 0.4 g/m^2/day.

Dissolved CH_4 values ranged between 2.4 and 87,624 ppm but this variation was not related to treatment effects (Figure 6). Values across cores and over time are highly variable until week 4, when most cores drop below 9,215 ppm. The high average of the surface + subsurface treatment after week 4 is driven by one core with concentrations of 67,522, 87,624, and 38,382 ppm in weeks 10, 14, and 20, and deleting this core drops the averages to 361, 166, and 232 ppm.

Linear regressions of CH_4 flux and dissolved CH_4 to test the effects of whether the core was wet at sampling conclusion and percolation rate were not significant. Each regression also included treatment and experiment day, to fix for time.

N_2O flux and dissolved content

N_2O flux rates ranged from below detection to 1.67 g/m^2/day, with both surface exposed treatments increasing significantly through week 10, while control and subsurface exposed rates remained low (Figure 6). In the baseline, average flux across cores was 0.03 g/m^2/day. Cores in both surface exposed treatments rose sharply to an average of 0.57 g/m^2/day in week 1, and they decline in the following weeks, all falling to below pre-treatment levels (<0.10 g/m^2/day) by week 20. The high variability of the surface + subsurface treatment in weeks 3, 4, and 10 is driven by one core with high rates over 0.77 g/m^2/day for those weeks. Dropping this single core reduces the averages to 0.33, 0.25, and 0.18 g/m^2/day, nearly identical (within 0.05 g/m^2/day) to the surface-exposed averages for those weeks.

Cumulative estimated N_2O fluxes over the experiment ranged from 3.84 to 1,145.8 mg over 4 months across the 20 cores, with both sets of surface exposed cores tending to have higher N_2O fluxes than control and subsurface exposed cores ($p=0.068$, $F_3=2.89$). The high average flux of subsurface exposed cores is driven by one core with high rates in weeks 3, 4, and 10, which gave that core an estimated total flux of 1,145.8 mg (the next lowest value is 403.4 mg). Dropping this core gives the subsurface exposed treatment an average of 192.6 mg, closer to the control average.

Dissolved N_2O concentrations were often below detection (~40% of all samples collected) and ranged as high as 304.3 ppm. Dissolved N_2O did not show significant treatment effects, and concentrations generally declined through time (repeated measures ANOVA sample event significance: $p=0.005$, $F_6 = 3.41$). Though the repeated measures ANOVA does not show a significant treatment effect over time, cores in both surface exposed treatments have significantly higher concentrations than control and subsurface exposed cores in weeks 1 and 2 ($p<0.05$), with the difference eliminated by week 3. Porewater N_2O was not measured for in the pre-treatment period or week 0, but significantly higher values in both surface exposed treatments in week 1 suggest a pattern similar to N_2O flux.

A linear regression of N_2O flux on whether the core was wet at sampling conclusion, treatment, and experiment day (to fix for time), indicates more fully saturated cores had 0.08 g/m^2/day lower N_2O flux

(adj. R-sq=0.16, p=0.049) than better drained cores. Using percolation rate shows an increase in N_2O production of 0.02 g/m^2/day for each 1 mm/hr increase in percolation rate (adj. R-sq=0.19, p=0.022). Similar regressions for N_2O concentration indicates wetter cores tended to have lower N_2O concentration in porewater than better draining cores (adj. R-sq=0.25, p=0.014,).

CO_2 flux and dissolved content

CO_2 flux values ranged from 5.8 to 510.0 g/m^2/hr and showed no significant treatment effects over time (Figure 6), or in estimated cumulative CO_2 flux, which ranged from 17.1 to 134.2 g per core over 4 months (Table 3). Average CO_2 flux in control and subsurface cores was generally higher than both surface exposed treatments, though individual rates greatly overlap between treatments, except in week 20, when subsurface cores are consistently higher than both surface exposed treatments ($p<0.05$), though not higher than control cores.

Dissolved CO_2 in porewater ranged from 4,490 to 114,474 ppm, with concentrations declining across all treatments through week 4 and then stabilizing (Figure 6; repeated measures ANOVA sample event significance: p=0.004, $F_6 = 3.56$). Rates show no significant treatment effects over time. Control cores tend to have higher values than other treatments, though rates greatly overlap between treatments.

A linear regression of CO_2 flux on whether the core was wet at sampling conclusion, treatment, and experiment day (to fix for time), indicates wet cores had 83.4 g/m^2/day lower CO_2 flux (adj. R-sq=0.18, $p<0.001$). Wetness and percolation rate had no effect on dissolved CO_2 concentrations.

Figure 6. Gas flux and dissolved gas, with repeated measures ANOVA F-statistic and p-value

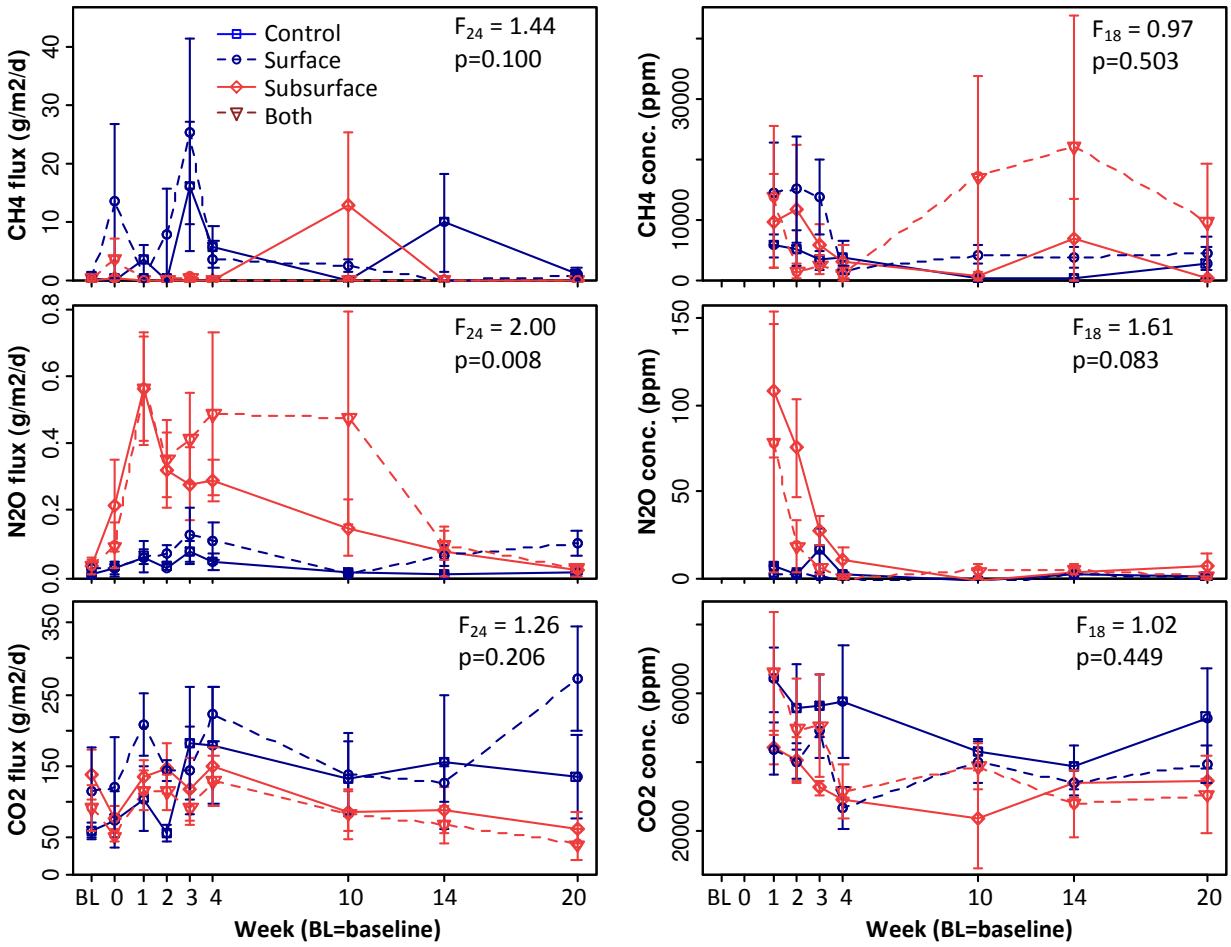


Table 3. Soil characteristics, iron, microbial data, and totaled gas flux. P-values for one-way ANOVA tests between treatments and means and standard deviations by treatment. Superscripts correspond to multiple comparison ANOVA results, which were run for variables with significant one-way ANOVA; values sharing letters are not significantly different.

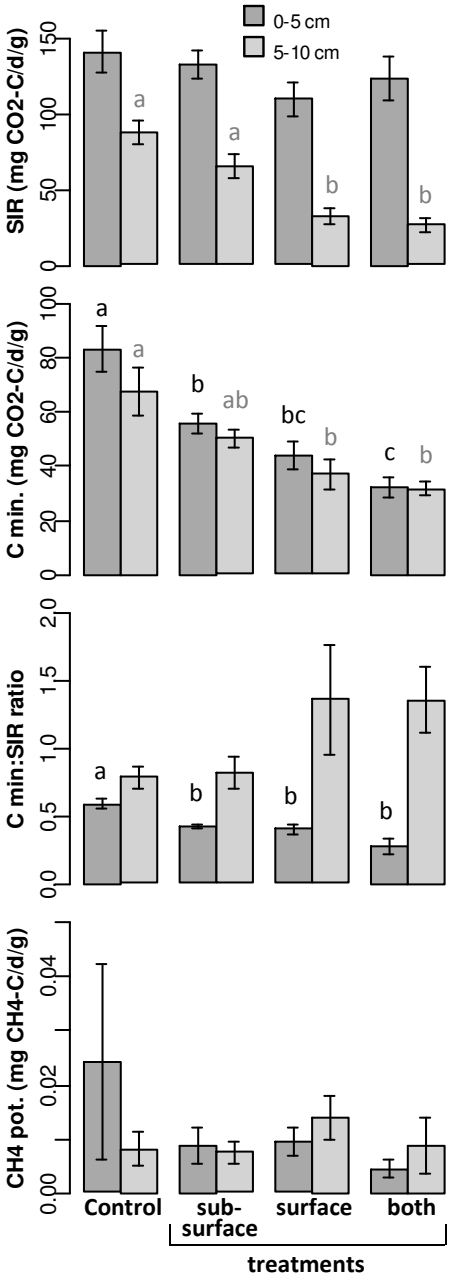
Variable	Depth (cm)	One-way ANOVA		Multiple Comparison ANOVA means, std. errors			
		p-value	F-stat (df=3)	Control	Subsurface	Surface	Both
Physical soil characteristics							
Percolation rates		0.088	2.60	0.08[a] ±0.01	0.11[ab] ±0.05	0.37[b] ±0.15	0.18[ab] ±0.04
Dry mass ratio (dry to total mass)	**0-5**	0.658	0.55	0.41 ±0.01	0.4 ±0.03	0.41 ±0.01	0.41 ±0.02
	5-10	0.232	1.59	0.44 ±0.01	0.47 ±0.02	0.51 ±0.04	0.49 ±0.02
AFDM portion	**0-5**	0.815	0.31	0.62 ±0.01	0.65 ±0.03	0.62 ±0.02	0.62 ±0.02
	5-10	0.700	0.53	0.63 ±0.02	0.66 ±0.03	0.6 ±0.06	0.65 ±0.02
Carbon mineralization (mg CO_2-C/d/g dry soil)	**0-5**	<0.001***	15.28	83.1[a] ±8.4	55.9[b] ±3.6	43.8[bc] ±5.1	32.3[c] ±3.8
	5-10	0.002**	8.11	67.6[a] ±8.9	50.2[ab] ±3.4	37.1[b] ±5.4	31.5[b] ±2.7
Iron							
Reduced iron (ppm)		0.001***	8.89	3.54[a] ±0.8	3.80[a] ±1.17	17.20[b] ±3.84	14.56[b] ±3.38
Microbial parameters							
Substrate induced resp. (mg CO_2-C/d/g dry soil)	**0-5**	0.353	1.16	141.3 ±13.7	132.7 ±8.9	110.4 ±11.2	123.8 ±14.5
	5-10	<0.001***	17.83	87.6[a] ±8.1	65.2[a] ±8.0	32.7[b] ±5.5	26.6[b] ±4.9
Methane potential (mg CH_4-C/d/g dry soil)	**0-5**	0.499	0.84	0.0242 ±0.0181	0.0088 ±0.0034	0.0045 ±0.0017	0.0096 ±0.0025
	5-10	0.629	0.59	0.0082 ±0.0032	0.0076 ±0.0020	0.0089 ±0.0051	0.0140 ±0.0041
Respiration efficiency (ratio of C min. to SIR)	**0-5**	<0.001***	9.59	0.591[a] ±0.041	0.422[b] ±0.013	0.404[b] ±0.038	0.283[b] ±0.059
	5-10	0.629	1.70	0.784 ±0.087	1.358 ±0.399	0.820 ±0.118	1.352 ±0.244
Estimated total gas flux over experiment							
Total CH_4 flux (mg)		0.505	0.81	2,374 ±1,391	3,064 ±1,799	1,492 ±1,404	261 ±212
Total CO_2flux (mg)		0.608	0.63	63,872 ±24,614	76,006 ±14,489	56,143 ±12,707	45,418 ±9,134
Total N_2O flux (mg)		0.068	2.89	15.9 ±7.3	28.7 ±7.6	117.2 ±30.7	383.3 ±61.6

Microbial Response

Microbial biomass by Substrate Induced Respiration

By the end of the experiment microbial biomass had dropped considerably from initial, day 0 estimates. SIR decreased with depth in all treatments, ranging from 67.5 to 190.6 mg CO_2-C/d/g dry soil in shallow 0-5 cm soil and 11.1 to 106.6 mg CO_2-C/d/g dry soil in 5-10 cm soil (Table 3, Figure 7A). Microbial biomass in 5-10 cm depth was significantly reduced in both surface salinity treatments, with average SIR in surface treatments 34% of average control SIR (Figure 7, $p<0.05$). The 0-5 cm soil depth had no significant treatment effects. All rates are considerably lower than rates at time zero, when average SIR in the 0-5 cm and 5-10 cm depth ranges was 768.5 and 341.4 mg CO_2-C/d/g dry soil respectively. The results suggest that live microbial biomass decreased by at least two-thirds in most cores by the experiment end, but SW-top and SW-both decreased further, especially in the 5-10 cm depth.

Figure 7A-D. Microbial response by depth and treatment. Different letters indicate significant differences (p<0.05)



Carbon mineralization

Carbon mineralization rates were less affected by soil depth, ranging from 22.2 to 107.7 mg CO_2-C/d/g dry soil in 0-5 cm soil, and 18.1 to 99.7 in 5-10 cm soil (Table 3, Figure 7B). Carbon mineralization was significantly lower at both depths in both surface salinity treatments relative to controls, and subsurface salinity cores were also lower in 0-5 cm soil (Figure 7B; $p<0.05$).

Respiration Efficiency

Respiration efficiency (carbon mineralization to SIR ratio) increased by depth in all treatments, ranging from 0.160 to 0.744 in 0-5 cm depth and 0.476 to 2.800 in 5-10 cm depth (Table 3, Figure 7C). Respiration efficiency in 0-5 cm depth was significantly lower in all salinity treatments relative to control. The 5-10 cm soil depth had no significant treatment effects.

Methane Potential

Methane potential ranged between 0.001 and 0.096 mg CH_4/d/g dry soil, but rates were unaffected by treatment and did not vary consistently with depth (Table 3, Figure 7D).

Discussion

Exposing coastal plain freshwater wetland sediments led to significant declines in microbial biomass and microbial carbon use efficiency and large increases in N_2O production. Contrary to predictions, we did not see a consistent suppression of CH_4 or stimulation of CO_2 as a result of treatment. We saw the expected decreases in DOC, caused by ions binding with dissolved carbon and flocculating it out of the porewater (Sholkovitz 1976), and significant increases in NH_4^+ concentrations, caused by ions displacing NH_4^+ from soil binding sites (Seitzinger 1991). Except for measures of microbial biomass and soil carbon mineralization, cores that received only subsurface salinity exposure were indistinguishable from control cores.

Cl^- concentrations in porewater rose considerably in both surface-exposed saltwater treatments, while subsurface-exposed cores remained at or near control levels. The soil cores had a thin layer of clay-silt sediment at the bottom which likely limited diffusion from groundwater. This is consistent with slow diffusion rates at lower depths observed in similar experiments by Weston et al (2009), where it took 3 months for Cl^- from surface amendments to reach sediment below 16 cm depth, and 1 year for the >16-cm depth sediment to reach the same salinity as the amendments. Evidence in the field also suggests that exchange between surface and groundwater in coastal wetlands is limited or slowed by clay or peat deposits (Howes et al 1996). While the lack of salinity impacts from subsurface treatment may suggest lesser biogeochemical impacts from subsurface intrusion, it may be a result of experiment design: pouring surface water amendments into the tops of cores may have prevented ions from diffusion upward.

By the experiment conclusion, microbial biomass had dropped dramatically in all cores, but was lower in both surface exposed saltwater treatments. The widespread reduction was likely from decreasing organic matter availability over time that occurs in a closed-system experiment lacking new inputs. The decrease in microbial biomass in surface exposed cores may have resulted from physiological stress to microbes. In addition to evidence of salinity stress of some freshwater microbes (Yan and Marschner 2012, Rysgaard et al 1999), H_2S produced by SO_4^{2-} reduction is toxic to many biomolecules (Wang and Chapman 1999). While studies do not reveal toxicity to methanogens—and this experiment documented no impact to CH_4 potential—much evidence exists for salinity or sulfide-induced stress and community composition shift of nitrogen-cycling microbes (Santoro 2010). In a natural system as opposed to a closed experiment, salinity-intolerant microbes lost from intrusion would potentially be replaced by salinity-tolerant microbes, lessening microbial biomass change.

Higher salinity sediments are typically associated with lower CH_4 production (Bartlett et al 1987), and the higher redox potential of SO_4^{2-} reduction is expected to suppress methanogenesis and CH_4 flux with saltwater intrusion, yet studies show mixed results. Though significant impacts were not seen in this experiment, it is notable that flux from both surface exposed treatments is consistently low, while flux in control and subsurface treatments is highly variable across cores and over time. Figure 7 also provides evidence for SO_4^{2-} reduction in both surface exposed treatments. While some experimental studies have clearly documented methanogenesis and CH_4 suppression (Weston et al 2006, Mishra et al 2003), another has shown an increase in CH_4 total flux and methanogenesis (Weston et al 2009). The authors

hypothesize that the increase in both SO_4^{2-} reduction and methanogenesis resulted from increased availability of dissolved organic matter as it's displaced by saltwater ions from mineral sorption sites. The significant reductions in DOC with saltwater addition observed in this study provide little support for this hypothesis. Additionally, methanotrophy is an important control on CH_4 emissions (Megonigal and Schlesinger 2002), and this process may be impacted as well, obscuring evidence of changes to methanogenesis.

Studies provide mixed findings on saltwater impacts to N_2O flux, though a 2010 literature review concludes that more evidence points to a decrease in nitrification and denitrification (Santoro 2010). Studies identify salinity and sulfide stress and toxicity to nitrifiers and denitrifiers as a potential cause for lower rates (Santoro 2010, Rysgaard et al 1999). Alternatively, some experimental studies have revealed higher nitrification rates at intermediate salinities, suggesting nitrifiers maximize efficiency when experiencing the average salinity of the source site (Bernhard et al 2007, Isnansetyo et al 2011, Magalhaes et al 2005). But it's important to note that these studies used brackish sediments already accustomed to a wide salinity range, likely fostering a microbial community tolerant to dynamic salinity. The sediment used in this experiment was from a freshwater wetland in an area with no documented exposure to saltwater, so it's unlikely higher salinity would increase efficiency of the microbial community. Additionally, microbial biomass was lower in saltwater exposed cores, suggesting a negative physiological response.

Lower NH_4^+ content in saltwater wetlands is often cited as another reason for lower nitrification rates in saltwater wetlands than in freshwater wetlands. Though it is well-documented that saltwater will lead to release of NH_4^+ from soil and subsequent export (Seitzinger 1991), it is unclear what the short-term implications of new saltwater encroachment are for nitrogen availability and nitrification. While Seitzinger (1991) proposed that dissolved and adsorbed NH_4^+ are equally available, Rysgaard et al (1999) found that nitrification was stimulated during a KCl-extraction of NH_4^+, which briefly raises dissolved NH_4^+. This suggests dissolved NH_4^+ is in fact more available to nitrifiers than adsorbed NH_4^+, meaning the salinity-induced increase in NH_4^+ concentrations in this experiment could have stimulated nitrification and fueled denitrification. The lack of NO_3^- increases suggests much of the NO_3^- produced must have been denitrified.

The increase in N_2O may have also been the result of a change in product composition of nitrification or denitrification, as opposed to a change in the denitrification rate. Nitrification is an overlooked source of N_2O in freshwater wetlands, and high NH_4^+ availability has been shown to elevate the level of N_2O produced from nitrification (Morse and Bernhardt 2013). In the current experiment, NH_4^+ may have become more available for nitrification when released from soil particles, leading to increased leakage of N_2O from nitrification. A shift in the N_2O:N_2 ratio from denitrification may have also caused the N_2O increase. Typically this ratio is small, but some studies suggest that saltwater wetlands have a higher N_2O:N_2 ratio than freshwater wetlands (Senga et al 2006, Tobias et al 2001), suggesting the salinity amendments could have led to replacement of N_2 with N_2O.

Rates of carbon mineralization and respiration efficiency represent the only instances where subsurface treatment impacts were seen: carbon mineralization is significantly lower in all treatments relative to the control, and though not significant, it is also lower in surface + subsurface exposed cores than in surface exposed cores. This result could indicate that less less labile carbon was remaining in these cores by the experiment conclusion in all saltwater amended cores—an explanation that would be consistent with a prior study that documented accelerated carbon mineralization, likely owing to the replacement of methanogenesis with more efficient sulfate reduction (Weston et al 2006, 2009). Such an explanation is however inconsistent with the lack of any significant CO_2 flux response to saltwater exposure in this experiment. Alternatively, lower carbon mineralization rates may reflect a less efficient microbial community, an explanation that is consistent with the reduction in microbial biomass seen in surface exposed cores.

Conclusion

This study found evidence for significant consequences of saltwater intrusion to element cycling and microbial communities. Previous literature suggested CH_4 suppression and CO_2 stimulation, though we found no evidence for either effect. Instead we saw an enhancement of N_2O production and hypothesize the cause to be release of NH_4^+ from ions in saltwater, suggesting that intrusion will result in pulses of N_2O from sediment until NH_4^+ is exported from the system. This stimulation of N_2O flux, which represented an 16-fold increase from control levels in cumulative emissions over the experiment, has implications for climate change, since N_2O is a greenhouse gas with 298 times the radiative forcing of CO_2 (IPCC 2007). The release of NH_4^+ also poses potential threats to local water quality, since excess NH_4^+not nitrified may flow into coastal waters, causing eutrophication. The release of NH_4 is likely to be especially strong in wetlands such as Timberlake that have an agricultural legacy and agriculture as an adjacent land use.

An important aspect to address in further research on saltwater intrusion is the set of transitional impacts when freshwater sediment is first exposed to saltwater. The experimental use of brackish sediments or freshwater sediments that have previously experienced intrusion events could obscure potential impacts, since past saltwater exposure would likely have fostered a more salinity-tolerant microbial community. It could also fail to capture potential nitrogen cycling impacts of NH_4^+ release, since past saltwater exposure may have already lowered NH_4^+ content. Future studies would also benefit from the use of saltwater treatments taken directly from seawater near the study site. Although the lower microbial biomass we found in saltwater cores provides evidence for salinity and H_2S intolerance of some microbe species, the decrease might be mitigated by the establishment of new species that saltwater intrusion would likely carry into freshwater wetlands.

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