

Hydrologic Context Alters Greenhouse Gas Feedbacks of Coastal Wetland Salinization

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ABSTRACT

Changes in sea-level rise and precipitation are altering patterns of coastal wetland hydrology and salinization. We conducted paired laboratory (20 weeks) and field (15 weeks) marine salt addition experiments to disentangle the effects of hydrology (permanent versus intermittent flooding) and elevated marine salts (sulfate versus other salt ions) on greenhouse gas (GHG) emissions from freshwater forested wetland soils. Marine salt additions strongly affected GHG emissions in both experiments, but the magnitude, and even the direction, of GHG responses depended on the hydrologic context in which marine salt exposure occurred. Under permanent flooding, carbon dioxide (CO₂) fluxes were unaffected by marine salts, whereas methane (CH₄) fluxes were significantly suppressed by the addition of sulfate (as K₂SO₄) both with and without marine salts. In contrast, in intermittently flooded field and laboratory soils elevated salinity reduced carbon mineralization and CO₂ fluxes, but enhanced CH₄ fluxes relative to both controls and treatments with

elevated sulfate. Thus, elevated salinity or alkalinity (and not sulfate) controlled both gaseous carbon fluxes under intermittent flooding. Nitrous oxide (N₂O) fluxes had contrasting responses in the field and laboratory. In the laboratory, N₂O fluxes were not significantly related to chemical treatment but increased with porewater ammonium concentrations, which increased in salinity treatments via cation exchange. In intermittently flooded field conditions, elevated salinity strongly suppressed N₂O fluxes because ammonium did not accumulate in porewater; it was likely lost through advection, dispersion, or plant uptake. Understanding dynamic hydrologic and vegetation patterns across wetland landscapes will be critical for predicting both the magnitude and direction of wetland GHG responses to increasing marine salt across broad spatial scales.

Key words: saltwater intrusion; sea-level rise; carbon; nitrogen; greenhouse gases; tidal wetland.

HIGHLIGHTS

- Marine salts suppress CO₂ and N₂O emissions in intermittently flooded wetland soils.
- Sulfate from marine salts suppresses CH₄ emissions in permanently flooded soils.
- Other salt ions shift soils from sinks to sources of CH₄ during intermittent flooding.

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INTRODUCTION

Coastal ecosystems are particularly vulnerable to climate change, through both sea-level rise and macroclimatic drivers such as changes in temperature and precipitation (Osland and others 2015). Collectively, these global changes alter both the flooding and salinity regimes of freshwater coastal wetlands. Sea-level rise increases inundation and salinity in historically freshwater wetlands (Herbert and others 2015; Craft 2012), but can also increase the extent of permanent and intermittent freshwater wetlands through the upstream extension of tidal influences (Ensign and Noe 2018). The frequency, timing, and intensity of droughts and storms can also alter the extent and timing of both wetland inundation and salinization (Michener and others 1997; Prigent and others 2007). At global scales, both wetland inundation and salinization (for example, Ringeval and others 2010; Kroeger and others 2017) are predicted to exert significant control on the greenhouse gas feedbacks of coastal wetlands, but we do not know the combined effect of these drivers across broad spatial or temporal scales (Stagg and others 2017). Because changes in salinity and hydrology are tightly linked, understanding how freshwater coastal wetlands respond to salinization under dynamic inundation regimes is critical for predicting broad-scale patterns of greenhouse gas emissions from coastal wetlands under future climate change.

Dynamic redox gradients, driven by the duration and frequency of surface water in wetlands and the abundance and growth rate of aerenchymous plants, have long been known to control the spatial and temporal patterns of microbial respiration and resulting greenhouse gas emissions within wetlands (Reddy and Delaune 2008; Mueller and others 2016). Carbon dioxide (CO₂) emissions are higher from well drained soils where oxic conditions allow aerobic processes to dominate. In contrast, methane (CH₄) emissions are higher under flooded, anoxic conditions where anaerobic processes, such as methanogenesis, dominate (Moore and Dalva 1993; Kelley and others 1995; Boon and others 1997; Yang and others 2013). Both aerobic (for example, nitrification) and anaerobic (for example, denitrification) processes produce nitrous oxide (N₂O) in wetland sediments. Emissions of N₂O from denitrification are generally stimulated by conditions that are suboptimal for denitrification, including low soil moisture that increases availability of oxygen for aerobic processes that are more energetically favorable (Seitzinger and others 2006). Production of N₂O from nitrification can

also be an important component of wetland N₂O emissions during drier, more oxic conditions (Ma and others 2008). Researchers have found widely varying responses of wetland N₂O emissions to flooding magnitude and frequency (for example, Freeman and others 1997; Morse and others 2012; Yang and others 2013).

Greenhouse gas feedbacks of coastal wetland salinization are difficult to predict, in part, because the consequences of marine salt exposure for carbon (C) and nitrogen (N) cycling may vary between oxidized and reduced sediments. The majority of previous research has focused on how marine salts alter rates of C cycling in wetland soils through elevated sulfate (SO₄²⁻) concentrations in reducing conditions. In anoxic soils, elevated SO₄²⁻ from seawater increases associated SO₄²⁻ reduction rates (Weston and others 2011). Elevated SO₄²⁻ reduction rates have been linked to increased soil C mineralization rates and associated CO₂ emissions from wetland soils (Weston and others 2011; Chambers and others 2011; Neubauer and others 2013), although CO₂ responses are not consistent across all salinization studies (Marton and others 2012; Herbert and others 2015). Increases in CO₂ fluxes associated with increased SO₄²⁻ reduction may be counterbalanced with reduced CH₄ emissions. Under anoxic conditions, SO₄²⁻ reducers typically outcompete methanogens for limited fermentation products (Megonigal and others 2003); thus, rates of methanogenesis and resulting CH₄ fluxes from wetland soils exposed to marine salts typically decrease (Marton and others 2012; Chambers and others 2011; Helton and others 2014). This predicted decline in CH₄ flux with salinization has been found in many but not all studies (see Weston and others 2011; Ardón and others 2018). Even though aerobic C mineralization can be 3 to 6 times higher than anaerobic C mineralization across a range of wetlands (Reddy and Delaune 2008), less is known about the response to elevated salinity in oxic sediments or under shifting redox conditions.

Marine salt enrichment may also alter microbial N processing rates and N₂O emissions from freshwater wetlands. Ammonium (NH₄⁺) released from soils during marine salt exposure through increased rates of cation exchange and/or C mineralization (Caraco and others 1989; Weston and others 2010; Ardón and others 2013; Steinmuller and Chambers 2017) can alter the availability of reactive N for nitrification and denitrification, which both produce N₂O. In addition, increases in sulfide (HS⁻) as a product of SO₄²⁻ reduction in marine salt exposed soils could prove particularly toxic to some

nitrifiers and denitrifiers (as reported by Joye and Hollibaugh 1995; Senga and others 2006), with the potential for soil salinization (and accompanying sulfidation) to reduce overall rates of nitrification and denitrification (Osborne and others 2015). Alternatively, increases in HS^- have been shown to stimulate denitrifying microbes capable of using HS^- as an electron donor (Brettar and Rheinheimer 1991; Burgin and others 2012). These contrasting stress vs. subsidy responses by denitrifiers to HS^- or the relative importance of denitrification vs. nitrification to N_2O production under shifting redox conditions could explain the variation in N_2O emission responses to saltwater exposure observed across different tidal river floodplain soils (for example, Marton and others 2012), although the interactions among these mechanisms across flooding regimes are not known.

To disentangle the potential effects of salinization and flooding regimes on C and N cycling in freshwater forested wetland soils, we conducted laboratory soil core experiments paired with an in situ marine salt addition experiment to answer the following questions: (1) What are the effects of marine salts on rates of greenhouse gas emissions and organic matter mineralization in freshwater wetland sediments? (2) To what extent are the effects of marine salts due to the addition of SO_4^{2-} versus other salt ions? And (3) Does the response to marine salts vary between intermittently and permanently flooded conditions?

Prior research at this study site shows CH_4 emissions in permanently flooded areas strongly declined with increasing SO_4^{2-} concentrations (Helton and others 2014). Thus, we predicted that elevated SO_4^{2-} concentrations would increase CO_2 and decrease CH_4 fluxes in permanently flooded soils where SO_4^{2-} reduction rates are high; but that the response would be lower in intermittently flooded soils where SO_4^{2-} reduction rates are lower. For N_2O emissions, there are potentially more complex responses; prior research at this study site shows (1) N_2O emissions are higher in drier soils since conditions for denitrification are suboptimal and N_2O emissions from nitrification are high (Morse and others 2012; Morse and Bernhardt 2013), (2) in permanently flooded soils, N_2O emissions are primarily driven by nitrate (NO_3^-) concentrations from agricultural runoff (Helton and others 2014), and (3) cation exchange during marine salt exposure increases NH_4^+ concentrations in surface and porewater (Ardón and others 2013). Thus, we predicted N_2O production from nitrification and coupled nitrification–denitrification would increase with available NH_4^+ ,

particularly in intermittently flooded soils where rates of nitrification are higher.

MATERIALS AND METHODS

Study Site

Our field site is a 440-ha restored forested wetland within the Timberlake Observatory for Wetland Restoration (TOWeR), a 1700-ha compensatory forested wetland mitigation site (Figure S1). The region, site, and the restoration practices have been described in detail by Ardón and others (2010) and Morse and others (2012); thus, we include a brief description here. TOWeR is located in the Outer Coastal Plain of North Carolina, USA, on the Albemarle Peninsula. The climate of the region is humid subtropical with a mean annual precipitation of 1330 mm y^{-1} and a mean annual temperature of 16.6°C (Ardón and others 2010). TOWeR drains to the Little Alligator River, which flows into the Alligator River and the Albemarle Sound. The Albemarle Sound is a seasonally brackish (0–7 ppt) microtidal system with wind-driven tides (Corbett and others 2007).

Like much of the Atlantic coastal plain of the USA, wetlands in the region were historically dominated by pocosins—nutrient-poor, freshwater, evergreen shrub and forest bogs (Richardson 1983). By the 1970s much of the landscape was cleared, drained, and converted to agriculture (Carter 1975). Currently, agriculture remains a dominant land use of the region, even though the majority of the area requires active pumping to prevent flooding of agricultural fields (Bhattachan and others 2018). As pumps are decommissioned, sea-levels rise, and wetland restoration practices continue, recovering and restored wetlands on former agricultural fields will continue to become an important part of the coastal plain landscape.

Restoration of the TOWeR agricultural area began in 2004 by lowering field crowns and filling drainage ditches and then planting 750,000 live saplings of obligate and facultative wetland tree species (that is, *Taxodium distichum*, *Nyssa sylvatica* var. *biflora*, *Nyssa aquatica*, *Fraxinus pennsylvanica*, *Salix nigra*, *Chamaecyparis thyoides*, *Quercus nigra*, *Quercus michauxii*, *Quercus phellos*, and *Quercus falcata* var. *pagodafolia*). In 2007, the former fields were hydrologically re-connected by disabling the downstream pump system, allowing the water table to rise and the site to be re-inundated. The site experiences wind-driven rather than astronomical tides, and this, together with its limited topographic relief, causes water to flow both downstream and

upstream as a result of either rainfall or strong winds off the Albermarle Sound, respectively.

Laboratory Soil Core Experiment

To isolate the effects of hydrology (intermittent versus permanently flooded) and salinity (SO_4^{2-} versus other salt ions), we designed a soil core experiment, modified from Ardón and others (2013). Forty-eight intact soil cores were collected on 24 June 2011 in an area 200 m from the site of the field salt addition experiment (Figure S1). Because the cores were collected from an area with relatively high elevation, these soils had not experienced elevated marine salts in the recent past. PVC pipes with sharpened edges (10 cm internal diameter and 40 cm deep) were pushed into the soil to collect an intact core that was approximately 35 cm deep and left approximately 5 cm of headspace above the soil surface. The cores were instrumented with a flexible tubing at the bottom to manipulate water level and a microlysimeter (RHIZON samplers, Netherlands) at 5 cm from the soil surface to collect porewater samples. Cores were maintained in the dark at 24°C and 25% humidity during the experiment.

After 4 weeks of equilibration, hydrology treatments were applied. All cores received watering once a week. In half of the cores the height of the flexible tubing was maintained so that water level inside the core was above the soil surface for the duration of the experiment (Permanently flooded, $n = 24$). In the other half of the cores, the flexible tubing was maintained at 25 cm below the soil surface, to allow drainage of the cores. In these cores the water level varied from dry (in between watering days) to standing water (immediately after watering) (Intermittently flooded, $n = 24$). To isolate the effects of SO_4^{2-} from other salt ions we modified an artificial seawater recipe (Kester and others 1967) to make the following treatments: (1) control (deionized water), (2) seawater (5 ppt), (3) saltwater (5 ppt seawater without SO_4^{2-}), and (4) SO_4^{2-} (as 4.0 g $\text{K}_2\text{SO}_4 \text{ L}^{-1}$ added to deionized water to achieve the same SO_4^{2-} concentration as the seawater treatment). We began adding the water chemistry treatments once a week on 16 August 2011 for 20 weeks.

We measured net soil-to-atmosphere greenhouse gas fluxes (CO_2 , CH_4 , N_2O) from the cores six times over the course of the experiment. On each sampling date a gas-tight lid with brass compression fittings and septa for gas sampling was placed on top of each core. Headspace gas samples were collected immediately and 2 h after capping the cores.

A pilot study confirmed that the assembly was gas tight and gases accumulated linearly during 2-h incubations. We measured the depth above the soil or water surface before each gas sampling date to calculate headspace volume. We analyzed gas samples and estimated fluxes as described in “Greenhouse Gas Analysis”.

We collected porewater after the hydrologic but before water chemistry treatments began on 6 July, and five times throughout the experiment corresponding with gas flux measurements. We connected a 5 ml syringe to each lysimeter, added a vacuum and left the syringes to collect soil solution for 12 h (Ardón and others 2013). Samples were collected in clean HDPE scintillation vials and analyzed immediately or frozen until analyses (within 3 weeks) for solutes as described below in “Water Chemistry Analysis”.

Field Marine Salt Addition Experiment

The experiment was conducted near the midpoint of the restored wetland (Figure S1). Surface water at the midpoint remained fresh (salinity < 0.08 ppt) for the duration of the experiment, and experienced moderate marine salt exposure in previous years with peak salinities of 4 ppt in 2007 and 2 ppt in 2008 and 2011 (Ardón and others 2013). Within a 3 by 9 m experimental plot, we installed 22 soil collars (15 cm tall by 20-cm-diameter PVC pipe) to a depth of 5 cm in the soil. We constructed boardwalks and a fence around the plot to minimize disturbance during collar installation and sampling. Collars were installed to avoid large plants, and salinity treatments were randomly assigned to collars. Five collars were each assigned the lowest (0 ppt) and highest (10 ppt) treatments. Three collars were assigned a treatment salinity of 4 ppt, the peak salinity of surface water measured at the midpoint during previous years (Ardón and others 2013). To one collar each, we assigned nine different treatments that spanned the range from the highest to lowest treatment salinity (0.5, 1, 2, 3, 5, 6, 7, 8, and 9 ppt).

Treatments were added weekly for 15 weeks (beginning 27 June 2012) by diluting concentrated seawater stock made in the laboratory (Kester and others 1967) with surface water collected at the midpoint location. Surface water was filtered through 1-mm mesh to remove algae and large organic debris and transported immediately to the experiment location. Each week we added 1.25 liters of treatment water at the appropriate concentration to each collar.

We measured net soil-to-atmosphere greenhouse gas fluxes (CO_2 , CH_4 , N_2O) on six dates from each of the 22 soil collars over the course of the experiment (132 total flux measurements). Gas flux measurements were initiated 2 h after we added treatments, using the static chamber approach (Livingston and Hutchinson 1995) following Morse and others (2012). Chamber tops were built from opaque 20-cm molded PVC caps fitted with gas-tight rubber gaskets, a 0.6 cm (one quarter inch) Swagelok brass sampling port with rubber septum, a vent tube, and an internal fan ($0.003 \text{ m}^3 \text{ s}^{-1}$; Jameco Electronics, Belmont, California, USA). We collected 10-ml headspace samples in triplicate with a glass syringe immediately following cap placement and after 1 and 2 h. Gas samples were injected into 9-ml pre-evacuated glass vials (Teledyne Tekmar, Mason, Ohio, USA). Air temperature and barometric pressure were recorded at the beginning of each sampling interval. Soil temperature at 5 cm, chamber height, and water depth were recorded immediately after the last gas sample was collected from each collar. We analyzed gas samples and estimated fluxes as described in “Greenhouse Gas Analysis”.

At the end of the experiment, we collected two duplicate 15-cm-deep soil cores from each collar using a 5-cm-diameter soil sampler with a slide hammer attachment on 1 November 2012, 3 weeks after the last gas flux measurements. A heavy rain (13 cm) resulting from Hurricane Sandy on 28 and 29 October immediately preceded our planned final soil core collection and inundated the experimental area. As a result, soil cores were collected with about 10 cm of overlying freshwater. Soil cores were immediately capped and transported to the laboratory on ice. We also collected surface water for use in subsequent soil process measurements. This water was transported to the laboratory on ice, filtered through $0.7 \mu\text{m}$ GF/F Whatman filters, and refrigerated prior to use.

Each soil core was separated into depth increments (0–5 cm and 5–10 cm), sieved (2 mm), and homogenized within 72 h. Duplicate soil cores were processed together to provide sufficient soil for all analyses. In this paper we report results from the 5–10 cm depth segment to limit the effects of surface flooding from Hurricane Sandy on the shallowest soil layer. Roots were extracted by hand during sieving and homogenization, dried in pre-weighed envelopes at 60°C and weighed on a Mettler Toledo PB precision balance (0.01 g). The sieved soil was used for all subsequent analyses. A 5–10 g soil subsample was weighed, dried at 60°C , and reweighed to determine soil water content

(SWC) ($\text{g water g dry soil}^{-1}$). The dried soil was then placed in a muffle furnace at 500°C for 1 h, and weighed again to determine ash free dry mass (AFDM) ($\text{g organic soil g dry soil}^{-1}$). Soil pH was measured with 3 g soil samples in 5 mL of 0.01 M CaCl_2 (Carter 1993). Dissolved NH_4^+ and NO_3^- were extracted from 2.5 g soil samples with 25 mL of 2 M KCl (Carter 1993). Water extractable solutes (DOC, TDN, SRP, SO_4^{2-} , Cl^- , NH_4^+ and NO_3^-) were determined by adding 2.5 g of field wet soil to 25 mL of DI water, placing the samples on a rotary shaker table at 150 rpm for 4 h, centrifuging the samples at 3400 rpm for 15 minutes, and filtering the supernatant through $0.7 \mu\text{m}$ GF/F Whatman filters. Concentrations in KCl and water extracts were analyzed as described in “Water Chemistry Analysis”.

We measured substrate-induced respiration (SIR) as an index of microbial activity (West and Sparling 1986) by adding 10 mL of a yeast and water solution to four grams of field moist soil in 40-ml sealed amber vials. Vials were placed at 150 rpm on a shaker table and we measured headspace CO_2 concentrations immediately after capping the vials and at 2 and 4 h. We measured C mineralization rates by adding 1 g of field wet soil and 10 mL filtered field water to a sealed serum vial. Headspace CO_2 concentrations were measured immediately after capping and after three and seven days. SIR and C mineralization headspace samples were run immediately on an LI-6250 CO_2 analyzer (Li-cor, Inc., Lincoln, Nebraska, USA). SIR and C mineralization rates ($\text{g CO}_2 \text{ d}^{-1} \text{ g dry soil}^{-1}$) were calculated as the slope of CO_2 accumulation in the headspace over time per g of soil, corrected for soil water content.

We conducted anoxic assays to measure methanogenesis potential by adding 5 g of field wet soil and 10 mL of filtered field water to a serum vial. Vials were evacuated and flushed with N_2 three times, and then filled with N_2 to ambient pressure. Initial 1-ml headspace gas samples and samples on days three and seven were collected and injected into 9 mL pre-evacuated glass vials (Teledyne Tekmar, Mason, Ohio, USA) diluted with 9 mL N_2 . Gas samples were analyzed on the gas chromatograph within 2 weeks of collection (see “Greenhouse Gas Analysis”). Potential methanogenesis rates ($\text{mg CH}_4 \text{ d}^{-1} \text{ g dry soil}^{-1}$) were calculated as the slope of CH_4 accumulation over time per g of soil, corrected for soil water content.

After 3 weeks, overlying water was sampled from the anoxic assays and analyzed for reduced iron (Fe^{2+}) according to Stookey (1970) and Gibbs

(1979) and HS^- using a modified methylene blue method (Cline 1969). Samples were kept in the dark and read at 562 nm (Fe^{2+}) and 663 nm (HS^-) on a LKB Biochrom Ultraspec II spectrophotometer (Biochrom, Holliston, Massachusetts, USA) within 1 week of collection.

Greenhouse Gas Analysis

Gas samples were analyzed within 2 weeks of collection as described in detail by Morse and others (2012). Briefly, gas samples were analyzed for CO_2 , N_2O , and CH_4 concentrations injected by a Tekmar 7050 Headspace Autosampler to a Shimadzu 17A gas chromatograph with electron capture detector (ECD) and flame ionization detector (FID; Shimadzu Scientific Instruments, Columbia, Maryland, USA), retrofitted with sixport valves and a methanizer to allow the determination of the three gases from the same sample.

We converted gas concentrations by volume (ppmv) to mass (mg m^{-3}) using the ideal gas law and field or laboratory measurements of barometric pressure and air temperature, and calculated gas fluxes as the slope of the simple linear regression between concentration and time. From replicate gas samples, we calculated the minimum detectable concentration difference for each sampling date (MDCD; Yates and others 2006; Matson and others 2009). For the field experiment, if the change in gas concentrations was greater than MDCD, then to estimate fluxes we used the slope of concentration versus time when r^2 is greater than 0.85. When the accumulation rate was nonlinear, and the change in concentration during the first interval was greater than MDCD, we used the rate during the first interval; when a longer incubation was needed to exceed MDCD, we used the rate calculated from the initial to the final sample. If the change in concentration was less than MDCD from the initial to the final sample, then the gas flux was set to zero (for the field experiment, $n = 2$ for CO_2 , 9 for CH_4 , and 14 for N_2O ; all fluxes were greater than MDCD for the laboratory experiment). Static chambers are sensitive to disturbance and chamber effects, and thus we excluded incubations with elevated initial concentrations and incubations in which the concentration increased significantly then decreased significantly, or vice versa ($n = 7$ for CO_2 , 13 for CH_4 , and 23 for N_2O).

Water Chemistry Analysis

Water samples and soil extracts were analyzed according to Standard Methods (APHA 1998). Concentrations of SRP (ascorbic acid method) and

NH_4^+ (phenate method) were analyzed on a Lachat QuikChem 8000 (Lachat Instruments, Milwaukee, Wisconsin, USA). Minimum detection limits were $5 \mu\text{g N L}^{-1}$ for NH_4^+ and $2.5 \mu\text{g L}^{-1}$ for SRP. Concentrations of NO_3^- , Cl^- and SO_4^{2-} were measured on a Dionex ICS-2000 ion chromatograph (Dionex Corporation, Sunnyvale, California, USA). Minimum detection limits were $5 \mu\text{g N L}^{-1}$ for NO_3^- , 0.03 mg L^{-1} for Cl^- , and 0.01 mg L^{-1} for SO_4^{2-} . TDN and DOC were measured on a Shimadzu TOC-V total carbon analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia, Maryland, USA). Minimum detection limits were 0.25 mg L^{-1} for DOC and 0.05 mg L^{-1} for TDN. Concentrations measured below detection were set to one half of the detection limit.

Data Analysis

Data analyses were performed in R 3.0.2 (R Core Team 2013). We calculated cumulative gas fluxes from both the field and laboratory experiments. First, we calculated daily gas fluxes by linearly interpolating gas fluxes between sampling dates, and then we calculated cumulative fluxes by summing daily fluxes over the course of each experiment (84 total days for the field experiment and 176 total days for the laboratory experiment). We used simple linear regression to analyze the relationships between measured parameters in the field experiment (cumulative gas fluxes, soil process rates, and soil chemistry) and field conditions (salinity treatment, average water depth, average soil temperature). We also used multiple linear regression selection to quantify relationships between cumulative gas fluxes and (1) field conditions (salinity treatment, average water depth, and average soils temperature), and (2) soil chemistry measured at the end of the experiment (water extractable SO_4^{2-} , SRP, NO_3^- , and NH_4^+ , AFDM, soil pH, Fe^{2+}). Multiple linear regression selection was performed with `regsubsets()` in the “leaps” package. `Regsubsets()` selects the best candidate model for each possible number of parameters from 2 to 8, including the intercept, with an exhaustive search that includes every combination of parameters at each level. We selected the best fit model as the model with the lowest Akaike’s information criterion (AIC). For the laboratory experiment, we used analysis of variance (ANOVA) followed by Tukey HSD post hoc tests to evaluate treatment effects on cumulative gas fluxes and porewater chemistry. For all parameters, normality was tested with the Shapiro–Wilk Normality Test and natural log transformed as indicated in the results to improve normality.

RESULTS

Treatment Chemistry

As intended, Cl^- and SO_4^{2-} concentrations in porewater collected from the laboratory experiment increased in treatments to which they were added, with Cl^- and SO_4^{2-} reaching concentrations as high as 7000 mg L^{-1} and 1200 mg L^{-1} , respectively, by the end of the experiment (Figure 1A–D). Similar to the laboratory experiment, water extractable Cl^- and SO_4^{2-} from the field experiment soils increased with marine salt treatments (Figure 1E, F), from an average of 0.11 and 0.005 in the lowest salinity treatments to 4.58 and $0.53 \text{ g kg dry soil}^{-1}$ in the highest salinity treatments, respectively. Although we did not measure pH in the laboratory experiment, marine salt enrichment also increased the soil pH from an average of 3.1 to 4.0 in the field addition experiment (Figure 2A).

Carbon Dioxide Emissions and Carbon Mineralization

In the laboratory experiment, cumulative fluxes of CO_2 tended to be higher from the intermittently

than the permanently flooded soil cores, but the relationship between CO_2 fluxes and chemical treatment depended on hydrologic treatment (Figure 3A, B). For the permanently flooded soil cores, cumulative CO_2 fluxes were not significantly different among chemical treatments (Figure 3A) while for the intermittently flooded cores, elevated salinity (both with and without SO_4^{2-}) significantly reduced cumulative CO_2 fluxes relative to the control and SO_4^{2-} treatments (Figure 3B). Contrary to our predictions, we did not find evidence for increased CO_2 fluxes when we added SO_4^{2-} alone. These findings suggest that net CO_2 fluxes are suppressed by elevated salinity under oxic or variable redox conditions, but are unaffected by elevated SO_4^{2-} even under reduced conditions where we expect higher rates of SO_4^{2-} reduction.

In the field, we also found evidence of suppression of CO_2 fluxes by marine salts in intermittently flooded soils. Net fluxes of CO_2 measured from our field plots ranged from 5 to $594 \text{ mg m}^{-2} \text{ h}^{-1}$ (mean \pm std; 123 ± 113) with cumulative fluxes ranging from 72 to 613 g m^{-2} (240 ± 170) over the course of the 15-week experiment (Figure 4A–

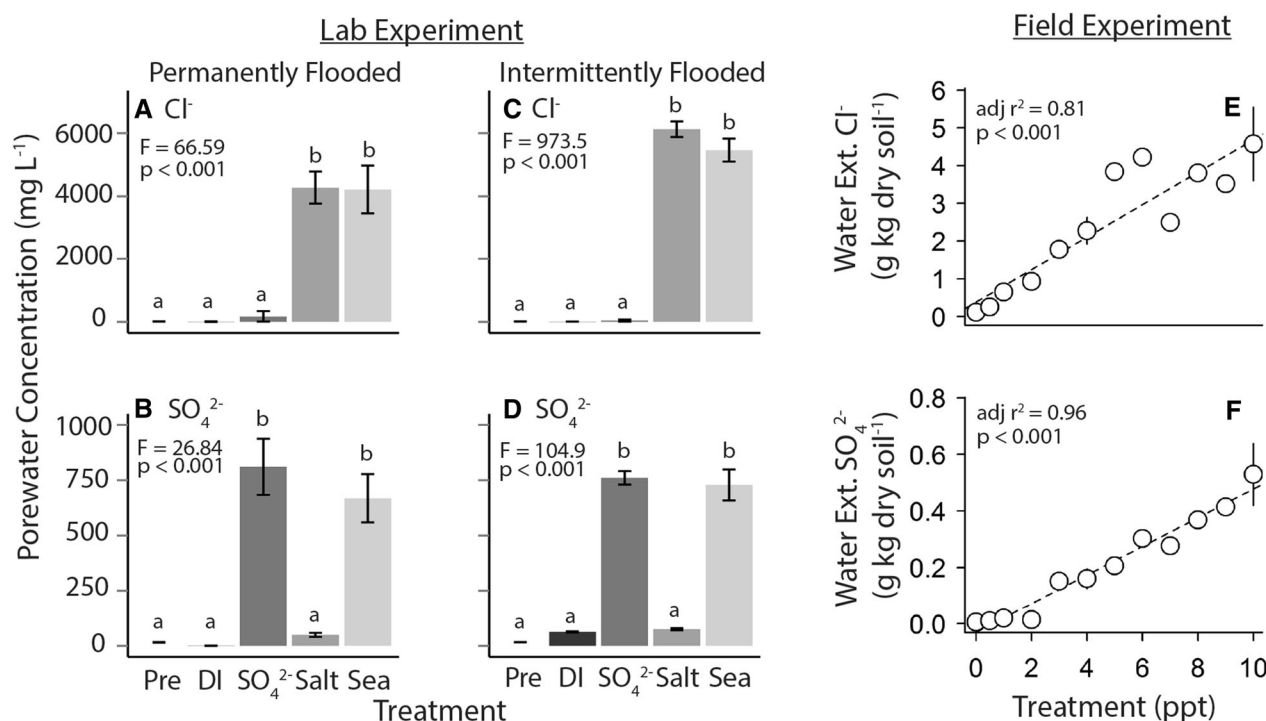


Figure 1. Porewater concentrations of Cl^- and SO_4^{2-} for permanently (A, B) and intermittently (C, D) flooded laboratory treatments for samples collected before ("Pre") and three months after chemical treatments were applied. Treatments are DI = control (deionized water), $\text{SO}_4^{2-} = 4.0 \text{ g K}_2\text{SO}_4 \text{ L}^{-1}$, Salt = 5 ppt seawater without SO_4^{2-} , Sea = 5 ppt seawater. Water extractable E Cl^- and F SO_4^{2-} for soils collected at the end of the field experiment. Field treatment salinities 0, 4, and 10 ppt plotted as mean \pm std. error.

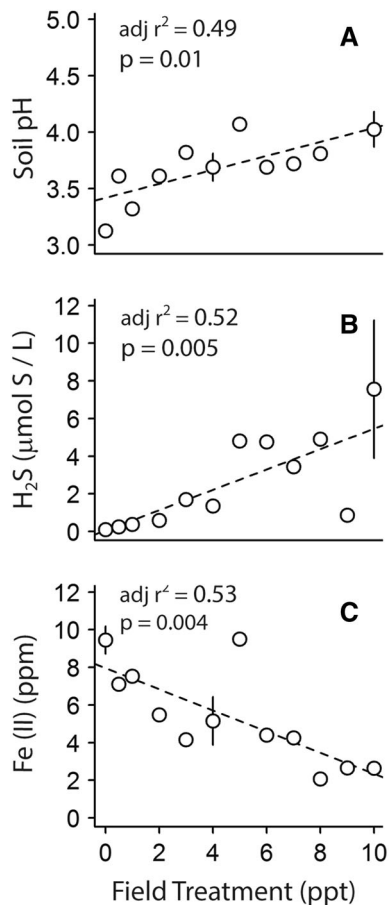


Figure 2. **A** Soil pH, and **B** HS^- and **C** Fe^{2+} concentrations for soils collected at the end of the field experiment. Field treatment salinities 0, 4, and 10 ppt plotted as mean \pm std. error.

D). Similar to the laboratory experiment, hydrology (average water depth) was a strong determinant of net cumulative CO_2 flux, with higher water depth suppressing CO_2 fluxes (Figure 4A). Although salinity treatment alone did not explain any of the observed variation in cumulative CO_2 fluxes (Figure 4C), after taking water depth and soil temperature into account, salinity treatment concentration significantly improved our multiple regression model and was negatively related to cumulative CO_2 fluxes (Table S1, Figure 4D).

When we brought these experimentally manipulated soils back to the laboratory and measured CO_2 fluxes under identical soil moisture and temperature, we found that field treatment salinity was strongly negatively correlated with soil CO_2 fluxes (Figure 5). Both SIR and C mineralization declined with increasing field treatment salinity (Figure 5A, B), with salinity treatment explaining 72 and 67%

of the variation in SIR and C mineralization, respectively. Cumulative CO_2 fluxes measured in the field were significantly but weakly related to C mineralization rates measured in the laboratory ($r^2 = 0.23$, $p = 0.02$) and were not related to SIR ($p = 0.11$). Even though there was strong evidence of significant SO_4^{2-} reduction through the accumulation of HS^- in C mineralization assays (Figure 2B), we did not observe enhanced CO_2 fluxes with increasing marine salt enrichment in the field experiments.

Methane Emissions and Methanogenesis Potential

In the laboratory experiment, salt treatments significantly affected CH_4 fluxes in both permanently and intermittently flooded soils, but the direction and magnitude of the responses depended on hydrologic treatment (Figure 3C, D). Permanently flooded cores typically had higher cumulative CH_4 fluxes (DI control mean \pm std; $0.91 \pm 0.73 \text{ g m}^{-2}$) than intermittently flooded cores ($-0.0042 \pm 0.0054 \text{ g m}^{-2}$). In permanently flooded cores, as we predicted, adding SO_4^{2-} reduced cumulative CH_4 fluxes relative to controls (Figure 3C). In contrast to our predictions, although cumulative CH_4 fluxes were typically much lower for intermittently flooded cores (note scale on y-axis in Figure 3D), cores receiving elevated salinity (with or without SO_4^{2-}) emitted significantly more CH_4 than controls or cores receiving SO_4^{2-} alone. Increasing salinity changed soils from net sinks to net sources of CH_4 .

We found these contrasting CH_4 flux responses to salinization in the field as well. During our field experiment in intermittently flooded soils, net CH_4 fluxes ranged from below detection to $8.1 \text{ mg m}^{-2} \text{ h}^{-1}$ (mean \pm std; 0.46 ± 0.96), and cumulative fluxes ranged from 0.26 to 3.77 g m^{-2} over 15 weeks (0.89 ± 0.90). Cumulative CH_4 fluxes were not related to average water depth (Figure 4E), but were weakly positively related to soil temperature and to field treatment salinity (Figure 4F, G). The best fit linear regression model included soil temperature and treatment salinity (Table S1; Figure 4H). In contrast to cumulative CH_4 fluxes measured in the field, methanogenesis potential rates measured under anoxic conditions in the laboratory from soils collected at the end of the field experiment strongly declined with treatment salinity (Figure 5C). Treatment salinity explained 75% of the variation in methanogenesis

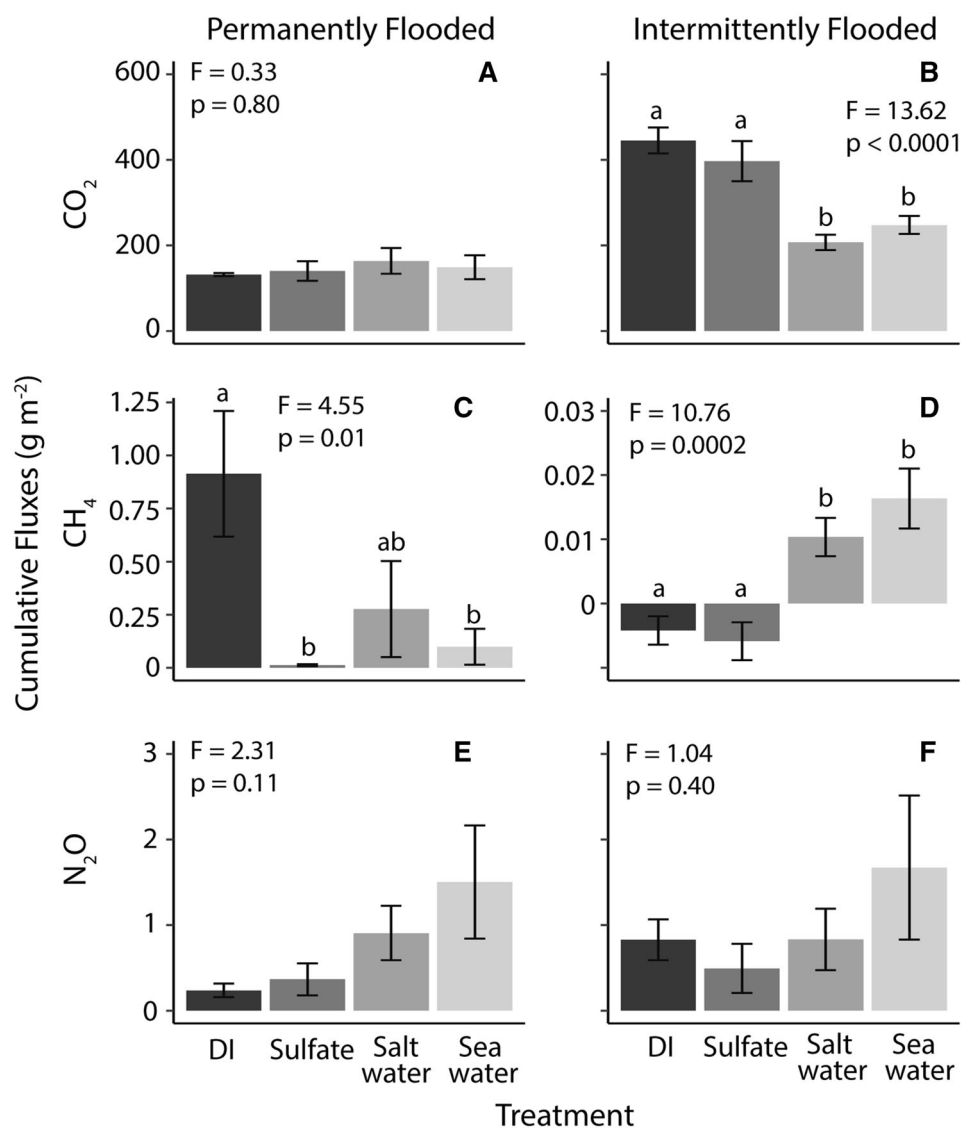


Figure 3. Laboratory experiment cumulative greenhouse gas fluxes for CO₂ (A, B), CH₄ (C, D), and N₂O (E, F). Treatments are DI = control (deionized water), SO₄²⁻ = 4.0 g K₂SO₄ L⁻¹, Salt = 5 ppt seawater without sulfate, Sea = 5 ppt seawater.

potential. Cumulative CH₄ fluxes measured in the field were not significantly related to methanogenesis potential measured in the laboratory ($p = 0.29$). From our previous research, we also found a strong decline in CH₄ emissions with SO₄²⁻ concentrations in permanently flooded areas in the field (Helton and others 2014). Together, these results suggest redox conditions in which wetland soils experience marine salt enrichment affect not only the magnitude but the direction of the CH₄ emission response, with elevated SO₄²⁻ reducing CH₄ emissions under anoxic conditions and other salt ions increasing CH₄ emissions under oxic or variable redox conditions.

Nitrous Oxide Emissions and Porewater Nitrogen Concentrations

In the laboratory experiment, the range and magnitude of cumulative N₂O fluxes were similar for permanently and intermittently flooded treatments and were not significantly different among water chemistry treatments (Figure 3E, F). Although not significant, the addition of marine salts tended to increase N₂O emissions relative to the control for both hydrologic treatments, a pattern opposite to that observed in our field experiments. Fluxes of N₂O measured during the field experiment ranged from -19 to $492 \mu\text{g m}^{-2} \text{h}^{-1}$ (mean \pm std; 30 ± 70), and cumulative fluxes ranged from $-$

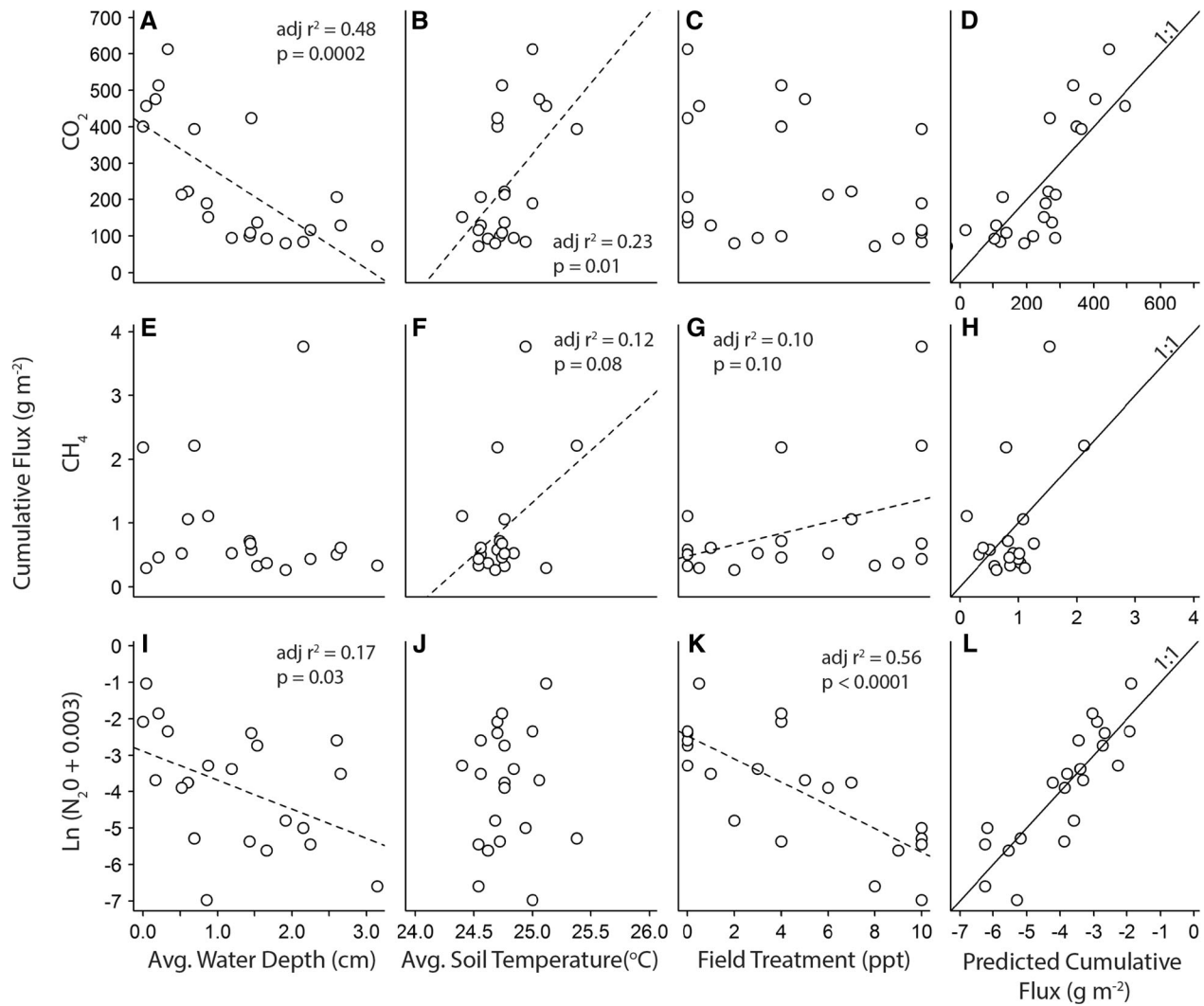


Figure 4. Field experiment cumulative greenhouse gas fluxes versus average water depth, average soil temperature, field salinity treatment, and predicted cumulative gas fluxes for CO₂ (A–D), CH₄ (E–H) and N₂O (I–L). Predicted cumulative gas fluxes are from the best fit multiple linear regression models (Table S1). **D** CO₂ = $-5478 - 93.4 (\text{wat. depth}) + 238.2 (\text{soil temp.}) - 14.0 (\text{treatment})$, $\text{adj } r^2 = 0.56$, $p < 0.001$. **H** CH₄ = $-32.4 + 1.3 (\text{soil temp.}) + 0.07 (\text{treatment})$, $\text{adj } r^2 = 0.15$, $p = 0.10$. **I** Ln(N₂O + 0.003) = $-1.7 - 0.30 (\text{treatment}) - 0.67 (\text{wat. depth})$, $\text{adj } r^2 = 0.71$, $p < 0.001$.

0.0021 to 0.35 g m⁻² (0.053 ± 0.082). In contrast to our predictions, cumulative N₂O fluxes in the field strongly declined with treatment salinity ($r^2 = 0.56$, Figure 4K). The best fit linear regression model that included water depth and treatment salinity explained 71% of variation in cumulative N₂O fluxes (Table S1; Figure 4L). We did not measure N₂O fluxes during assays of soils collected at the end of the experiment.

Patterns of porewater N concentrations may help explain the discrepancy among field and laboratory results. We measured significant increases in soil solution NH₄⁺ concentrations in the laboratory experiment (Figure 6A, C) and salinity decreased soil-bound NH₄⁺ at the end of the field experiment

(solid circles, Figure 6E), suggesting elevated salinity caused cation exchange and reduced soil-bound NH₄⁺ in both the field and the laboratory as has been documented extensively at our study site (Ardón and others 2013). However, exchangeable NH₄⁺ did not accumulate in porewater in the field since water extractable NH₄⁺ concentrations were not related to salinity treatment (open circles, Figure 6E). Thus, experimental conditions may artificially increase N availability to microbes that would otherwise be lost from system through advection, dispersion, or plant uptake under field conditions.

Concentrations of NO₃⁻ also varied among field and laboratory experiments, but in a pattern opposite to that of NH₄⁺. In the laboratory, we

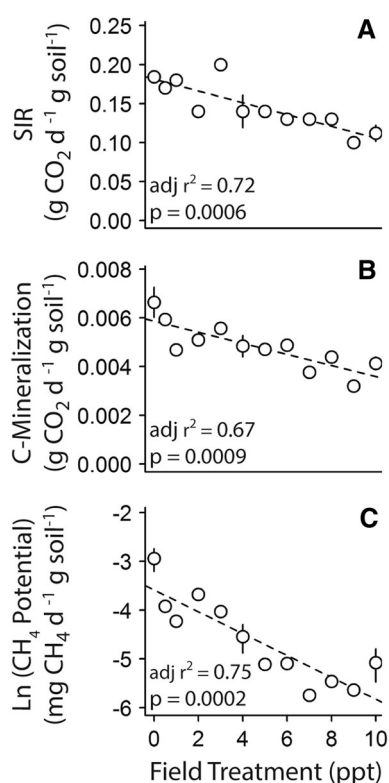


Figure 5. Soil process rates for soils collected at the end of the field experiment: **A** substrate-induced respiration (SIR), **B** carbon mineralization, and **C** methanogenesis potential. Field treatment salinities of 0, 4, and 10 ppt plotted as mean \pm std. error.

found adding SO_4^{2-} alone to intermittently flooded soils increased NO_3^- concentrations (Figure 6D), with NO_3^- accumulating to more than $50 \times$ higher than typical porewater concentrations measured in the field at our site (Ardón and others 2013; Helton and others 2015). Similarly, in the field we found KCl extractable NO_3^- was higher with elevated salinity (solid circles, Figure 6F). Water extractable NO_3^- was not related to treatment salinity (open circles, Figure 6F).

Relationships Between Greenhouse Gases and Soil Chemistry

To explore the relationships between cumulative greenhouse gas fluxes from the field experiment and soil chemistry, we performed an additional multiple linear regression model selection using a subset of soil chemical variables measured from soils collected at the end of the experiment (water extractable SO_4^{2-} , NH_4^+ , NO_3^- , SRP, and Fe^{2+} , soil pH, and AFDM) in place of treatment salinity (Table S2; Table 1). For cumulative CO_2 flux, the best fit model explained 76% of the variation and

included water depth, Fe^{2+} concentration, and AFDM. AFDM was not significantly related to treatment salinity (Figure S2), but Fe^{2+} concentrations declined strongly with treatment salinity (Figure 2C). Cumulative CO_2 flux significantly increased with Fe^{2+} concentration (adj $r^2 = 0.39$, $p = 0.002$).

Relationships between cumulative CH_4 flux and soil chemistry were less clear; the best fit model explained 56% of variation and included water extractable nutrients (NH_4^+ , NO_3^- , and SRP) (Table S2; Table 1). Water extractable NH_4^+ , NO_3^- , and SRP were not significantly related to field salt treatment, although SRP tended to peak at intermediate salinity treatment concentrations (Figure S2). Cumulative CH_4 flux also increased with SO_4^{2-} concentration (adj $r^2 = 0.16$, $p = 0.07$), but SO_4^{2-} was not included in best fit model (Table S2).

For cumulative N_2O flux the best fit model explained 69% of variation, and the relationships were similar to those found in the prior multiple linear model regression selection (Figure 4L). Water extractable SO_4^{2-} alone explained 50% of the variation in cumulative N_2O flux, and the best fit model included water depth and SO_4^{2-} concentration (Table S2; Table 1). The best fit model did not include water extractable inorganic N concentrations (Table S2).

DISCUSSION

Our results show that elevated marine salts strongly affect greenhouse gas emissions from freshwater forested wetland soils but the magnitude, and even the direction, of the response depends on the experimental and hydrologic context in which marine salt exposure occurs. Our results highlight three responses that contrasted expected patterns and could have major implications for how we predict greenhouse gas responses of freshwater wetlands to salinization: (1) Contrary to our predictions, we found that marine salts did not increase CO_2 fluxes in permanently flooded soils and suppressed CO_2 fluxes in intermittently flooded soils, with marine salt enrichment reducing CO_2 fluxes by an average of 40 and 45% in field and laboratory experiments, respectively. (2) As predicted and similar to the majority of previous research, CH_4 emissions were suppressed by marine salts in flooded soils; however, in contrast to our predictions, CH_4 emissions increased in intermittently flooded conditions, shifting wetland soils from sinks to sources of CH_4 in the laboratory, and more than doubling CH_4 emissions in the field. (3)

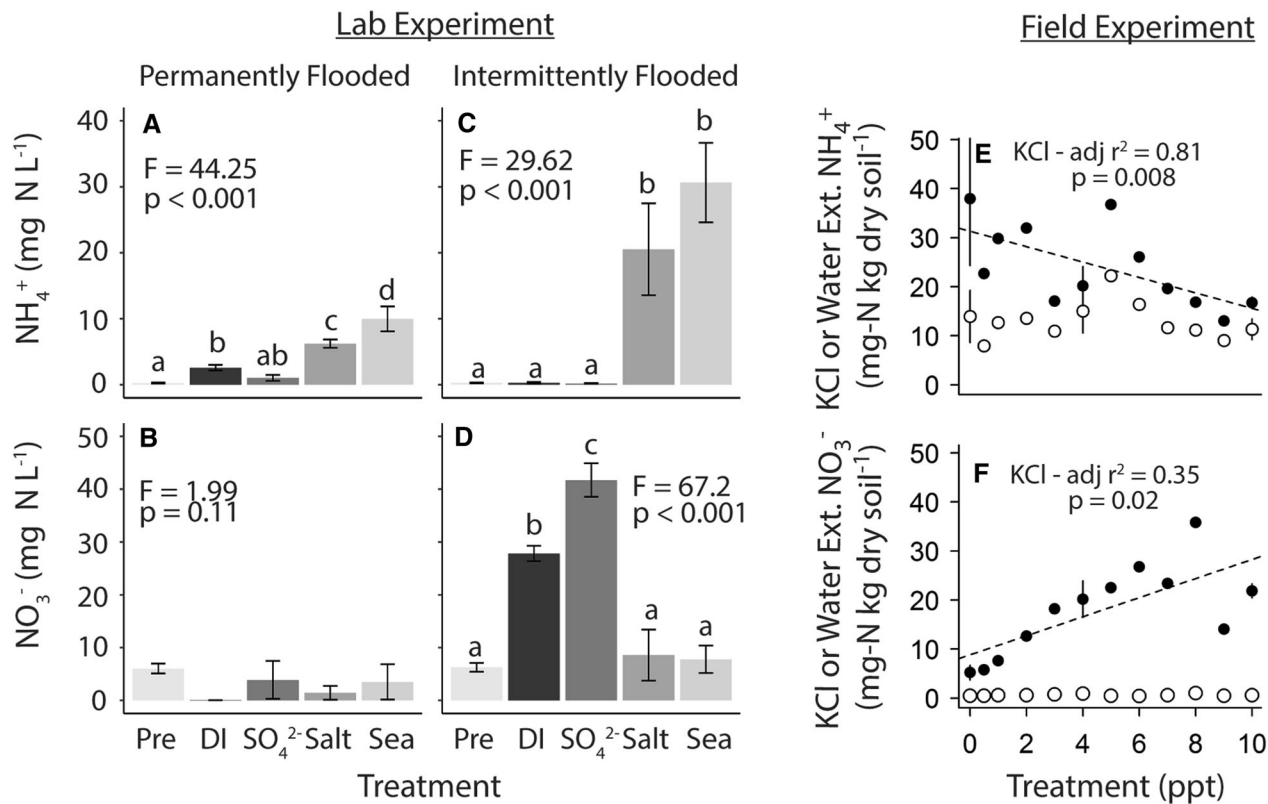


Figure 6. Porewater concentrations of NH_4^+ and NO_3^- for permanently (A, B) and intermittently (C, D) flooded laboratory treatments for samples collected before (“Pre”) and three months after chemical treatments were applied. Treatments are DI = control (deionized water), $\text{SO}_4^{2-} = 4.0 \text{ g K}_2\text{SO}_4 \text{ L}^{-1}$, Salt = 5 ppt seawater without SO_4^{2-} , Sea = 5 ppt seawater. Water extractable E NH_4^+ and F NO_3^- for soils collected at the end of the field experiment. Field treatment salinities 0, 4, and 10 ppt plotted as mean \pm std. error.

Table 1. Multiple Linear Regression Coefficient Estimates for Model with Lowest AIC for Cumulative Greenhouse Gas Fluxes From Field Experiment

Model	CO_2	CH_4	N_2O
Intercept	– 175.4	1.72	– 1.95
Wat. depth	– 102.7		– 0.79
SO_4^{2-}			– 0.004
SRP		0.20	
NH_4^+		– 0.17	
NO_3^-		– 1.56	
Fe^{2+}	26.3		
AFDM	842.1		
n	19	16	18
Adj. r^2	0.76	0.56	0.69
p value	< 0.001	0.005	< 0.001

See Supplemental Table 2 for full list of candidate models. Bold indicates significant coefficient at $p < 0.05$. SO_4^{2-} , SRP, DOC, NH_4^+ and NO_3^- are water extractions. Cumulative N_2O flux was Ln transformed before analysis.

Even though N_2O emissions increased under laboratory conditions with elevated marine salts as we

predicted, N_2O emissions were substantially lower and strongly declined in the field experiments, decreasing by 98% with marine salts in intermittently flooded soils. In the following, we explore potential mechanisms and implications of each of these responses.

Marine Salts Suppress Wetland Soil CO_2 Emissions

Results from both our field and laboratory experiments suggest elevated salinity suppresses net CO_2 fluxes from freshwater forested wetland soils with intermittent flooding regimes. This is in contrast to the expectation that elevated SO_4^{2-} in sea salts will enhance C mineralization through increased rates of SO_4^{2-} reduction (Chambers and others 2011; Morrissey and others 2014). Our laboratory experiment was designed to disentangle the effects of SO_4^{2-} versus other salt ions, and results suggest that the soil respiration response in intermittently flooded soils is driven by elevated salinity and not elevated SO_4^{2-} . Soil microbial communities may be

directly affected by elevated salinity, which can suppress rates of C cycling by reducing microbial biomass and respiration rates (Rath and Rousk 2015). Indeed, we observed a strong decline in SIR (a surrogate measure of microbial biomass) in soils collected at the end of our field experiment (Figure 5A), and wetland field experiments that increased salinity with NaCl rather than marine salts also tend to also report decreases in CO₂ emissions (Luo and others 2017). To maintain homeostasis with increasing external salt concentrations, microbes must use energetically expensive osmoregulation strategies, diverting resources from growth and survival (Schimel and others 2007; Rath and Rousk 2015). The response of microbes to elevated salinity can be exacerbated by low soil moisture (Rath and others 2017); thus, as our results suggest we may expect to see a stronger salt-induced suppression of microbial respiration in intermittently flooded soils.

In contrast to the majority of prior research (Herbert and others 2015; Luo and others 2017), we did not see an enhancement of CO₂ fluxes for treatments with added SO₄²⁻ alone (without other salt ions) for either permanently or intermittently flooded soil cores. We did find evidence of enhanced SO₄²⁻ reduction potential in field experiment soils under laboratory conditions (Figure 2B). This however did not lead to increased rates of CO₂ flux from field plots or experimental cores with elevated SO₄²⁻, even without other elevated salt ions. We hypothesize that sulfide may decrease CO₂ production through indirect effects on linked C and iron cycling. The microbially mediated reduction of iron(III) oxides (Fe³⁺) to Fe²⁺ is coupled to the oxidation of organic C to CO₂. Iron reduction can be a major organic C mineralization pathway in freshwater wetlands (Neubauer and others 2005). The abiotic reductive dissolution of Fe³⁺ with HS⁻ proceeds at a relatively fast rate to form iron sulfide (FeS; dos Santos Afonso and Stumm 1992). Indeed, the ratio of microbial to abiotic Fe³⁺ reduction decreases seaward as HS⁻ availability for abiotic Fe³⁺ reduction increases (Luo and others 2016). Our multiple linear regression analysis suggests that CO₂ fluxes are higher where more Fe²⁺ is available and previous research at our field site suggests high rates of Fe³⁺ reduction associated with locations with elevated salinity and higher rates of SO₄²⁻ reduction (Schoepfer and others 2014). Thus, the likelihood of increased SO₄²⁻ reduction increasing overall organic C mineralization rates may depend upon the dominance of microbial Fe³⁺ reduction within wetland soils.

Intermittently Flooded Soils are CH₄ Sources with Salinization

Our results suggest that the response of CH₄ to marine salt enrichment is strongly determined by the redox conditions in which soils experience elevated marine salts. Suppression of CH₄ emissions by SO₄²⁻ occurred in anoxic laboratory conditions, in permanently inundated experimental soil cores, and in permanently inundated field locations in a previous study at the same forested wetland (Helton and others 2014). This agrees with the majority of previous research (Luo and others 2017) and expectations that in the absence of oxygen, SO₄²⁻ reducers outcompete methanogens for limited fermentation products, suppressing CH₄ production (Megonigal and others 2003). In contrast, CH₄ emissions from intermittently flooded soils significantly increased with saltwater exposure (with or without elevated SO₄²⁻) in our laboratory and field experiments. In intermittently flooded soils, the availability of oxygen in soil surface layers was likely high enough to suppress methanogenesis (regardless of the presence or absence of SO₄²⁻) and rates of methanotrophy were high enough to consume most CH₄ produced; in our study intermittently flooded soils were often a sink for CH₄ when salinity was low but always a CH₄ source when salinity was high.

We hypothesize that there are several potential mechanisms that could explain increases in CH₄ emissions with salinization: (1) Methanotrophic bacteria that consume CH₄ may be more sensitive to salinity than methanogens, with the potential for differential salinity tolerance to generate significant increases in net CH₄ fluxes at ecosystem scales (Conrad 1995; Segers 1998); (2) Humic substances precipitate in the presence of multivalent cations (Tipping and Woof 1991), such as those found in seawater. Because microbial reduction in humic acids may competitively suppress acetoclastic methanogenesis and reduce CH₄ production (Keller and Bridgman 2007; Keller and Takagi 2013), humic precipitation induced by elevated marine salts may release methanogens and increase net CH₄ flux, particularly in intermittently flooded wetland soils where the competitive effects of SO₄²⁻ reduction on methanogenesis are likely lower (Ardón and others 2018); (3) Across ecosystems, a wide range of N enrichment increases CH₄ emissions by an average of 95% (30–400 kg N ha⁻¹ y⁻¹) and decreases methanotrophy by an average of 38% (10–560 kg N ha⁻¹ y⁻¹) (Liu and Greaver 2009). From Ardón and others 2013 we estimate that an additional 30 to 80 kg N ha⁻¹ y⁻¹

are released as NH_4^+ through cation exchange during saltwater intrusion from the site. Thus, changes in N availability from the release of soil-bound NH_4^+ by cation exchange (Ardón and others 2013) could increase net CH_4 fluxes from wetland soils.

Overall, our results suggest that increases in SO_4^{2-} reduction associated with marine salt enrichment may have little effect or even increase net CH_4 flux in areas where forested wetlands are intermittently flooded. Thus, scaling the effects of marine salts on CH_4 production measured in the laboratory or in permanently flooded field plots may overestimate the reduction of CH_4 emissions during salinization. Understanding dynamic hydrologic patterns across wetland landscapes will be critical for predicting the wetland CH_4 response to marine salt enrichment across broad spatial scales.

Response of N_2O Emissions to Marine Salts Differs in the Laboratory and Field

Our results suggest that the magnitude and direction of N_2O responses to marine salt depends on whether the response is measured under field or laboratory conditions. In agreement with our predictions and previous research (Ardón and others 2013; Zhou and others 2017), in the laboratory experiment N_2O emissions increased with elevated salinity along with the accumulation of NH_4^+ in porewater. In contrast to our predictions and our laboratory experiment, in the field experiment there was a strong decline in N_2O emissions with elevated marine salts.

We hypothesize that laboratory conditions could lead to the enhancement of N_2O emissions by changing N and C availability through three potential mechanisms. First, NH_4^+ may accumulate and concentrate in experimental cores simply because of the absence of hydrologic advection and dispersion, artificially increasing NH_4^+ availability to microbes that produce N_2O . Second, NH_4^+ may also have accumulated because of the lack of plant uptake in the laboratory experiment. In an earlier study using some of the same herbaceous freshwater wetland plants found at the TOWeR site, Sutter and others (2014) found that plant tissue N content significantly increased in response to experimental marine salt enrichment of 2 to 6 ppt. Research from other sites has also shown that plants can take up excess NH_4^+ to balance osmotic pressure under salt stress (Storey and Wyn Jones 1977; Munns 2002). Increased uptake of N by plants, in addition to hydrologic dispersion or

advection of available N, could reduce N availability for nitrification and coupled nitrification–denitrification in the field, potentially decreasing in situ N_2O emissions with elevated salinity.

Finally, microbes in laboratory soil core experiments are likely to be significantly more carbon limited than would be true under field conditions, given the absence of hydrologic exchange or plants supply of DOC. Plant root exudates can be a major source of labile carbon to heterotrophic microbes (Bertin and others 2003). Because nitrification is chemoautotrophic and the dominant pathway of N_2O production in this wetland (Morse and Bernhardt 2013), nitrifier use of available oxygen may have been enhanced in the laboratory where the more thermodynamically favorable oxidation of organic C was limited by the supply of labile C to heterotrophs. We hypothesize that under realistic scenarios of initial salinization, NH_4^+ released from cation exchange sites by marine salts is more likely to be accessed by plants than by nitrifiers. Under conditions of chronic salinity, high mortality of freshwater wetland plants could substantially reduce plant N demand and alter the fate of displaced NH_4^+ . Although we can only speculate from our current datasets, future experiments should consider how vegetation status and hydrologic context will interact to determine how N cycling will shift in response to salinization.

Marine Salt Exposure Across Ecosystem Types and Over Longer Time Horizons

Our study focused on short-term (weeks to months) response to moderate (0–10 ppt) marine salt exposure in a freshwater forested wetland. Over the short-term saltwater intrusion increases SO_4^{2-} concentrations and ionic strength, which mobilizes NH_4^+ , PO_4^{3-} , and trace metals (Herbert and others 2015). These changes have immediate effects on soil C and N cycling, as addressed in our study, which may vary across hydrologic conditions and ecosystem types. Indeed, variable responses of greenhouse gas emissions to elevated marine salts have been reported in other short-term intact soil core experiments. In peat cores from a mangrove forest, Chambers and others (2014) report an increase in CO_2 fluxes with elevated salinity (opposite to our findings, Figure 3), but only under simulated tidal hydrology and not for permanently flooded soils. In flooded, anoxic experiments with freshwater tidal marsh soils, Weston and others (2006) found that CH_4 fluxes decreased and C mineralization increased with marine salt exposure. In contrast, for freshwater tidal marsh soils

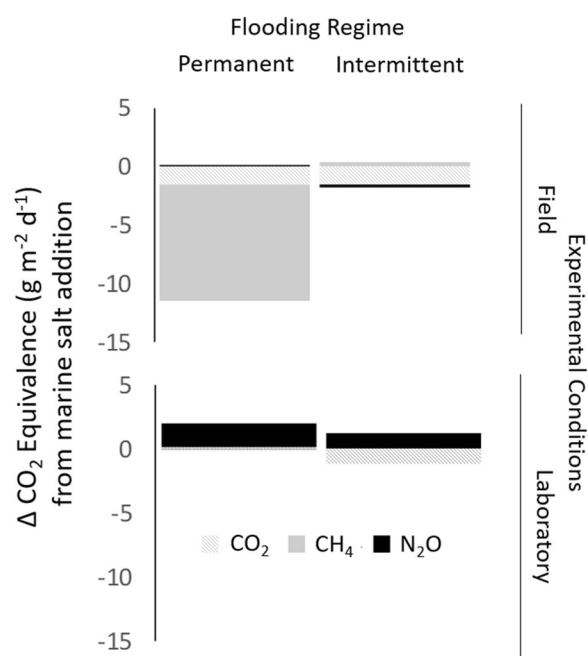


Figure 7. The change in greenhouse gas feedbacks from elevated marine salts in permanently and intermittently flooded soils in the field and in the laboratory. Field greenhouse gas responses for permanently flooded soils are reported in Helton and others (2014).

with simulated tidal hydrology, Weston and others (2011) found both CO₂ and CH₄ fluxes increased with elevated marine salts. In our forested freshwater wetland soils, we found a similar response for CH₄ fluxes (decrease under permanently flooded but increased under intermittently flooded conditions, Figure 3) but not for CO₂ fluxes (decreased under intermittently flooded conditions, Figure 3). There have been recent reviews of C and N cycling response to saltwater intrusion (Herbert and others 2015; Luo and others 2017; Zhou and others 2017) that highlight divergent short-term responses, but how and why these responses may vary among different wetlands types and hydrologic regimes is not well understood. Experimental design (such as choice of salt ions used) as well as factors that vary within and among wetland types, including salinity, soil properties, flooding regimes, plant characteristics, and land use history may all be important in determining the response of C and N cycles to saltwater intrusion. The divergent biogeochemical responses observed across experimental studies to date make it clear that predicting broad-scale effects of saltwater intrusion on greenhouse gas emissions will require a more explicit consideration of these complex drivers across ecosystems.

Although cases of wetland salinization from seawater were first reported more than three dec-

ades ago and have been documented extensively since that time (35 cases reviewed by Herbert and others 2015), relatively little is known about the long-term (years) implications of this gradual or episodic salinization trajectory for C and N cycling. Over longer time-scales, changes in plant productivity and species composition can have potentially large feedbacks to C and N cycling (Herbert and others 2015; White and Kaplan 2017), which may be divergent from short-term responses (Neubauer and others 2013). Reductions in productivity and changes in species composition can alter the amount and quality of organic matter added to wetland soils (Neubauer 2013; Pierfelice and others 2015; Stagg and others 2018). With chronic or long-term saltwater exposure, fresh and brackish wetland vegetation will eventually be replaced with salt marsh vegetation or open water (Herbert and others 2015; White and Kaplan 2017), which suggests that understanding the controls of C and N cycling along landscape salinity gradients is important for predictions under future scenarios (Stagg and others 2018). Expanding both our spatial and temporal understanding of the response of C and N cycling to marine salt exposure will be critical for predicting wetland ecosystem function in the future.

Elevated Marine Salts in the Context of Dynamic Landscapes

Our results clearly show that environmental settings—for example, redox condition or the presence of vegetation—control greenhouse gas feedbacks from freshwater coastal wetlands with marine salt enrichment. The net effect of salinization in both permanently and intermittently flooded wetlands under field conditions is a reduction in total global warming potential; however, the reduction in permanently flooded wetlands is approximately nine times higher than in intermittently flooded wetlands (Figure 7). The mechanism controlling the dominant greenhouse gas response also varied with environmental conditions; sulfate was the dominant driver of the C gas response in permanently flooded soils whereas salinity was the dominant driver of the C gas response in intermittently flooded soils. Successful upscaling and forecasting of freshwater coastal ecosystem greenhouse gas feedbacks to climate change will require the integration of hydrologic and ecosystem models that can adequately represent these heterogeneous, redox dominated landscapes. As broad-scale climate mitigation strategies that include greenhouse gas reductions from wetland flooding and salin-

ization are beginning to be proposed (for example, Kroeger and others 2017), our results highlight the need to consider dynamic hydrology across wetland landscapes when considering large-scale biogeochemical responses to marine salt enrichment.

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