Title: Greenhouse gas fluxes from coastal wetlands at the intersection of urban pollution and saltwater intrusion: a soil core experiment

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Highlights:

- Elevated sea salts and copper suppress carbon gas emissions from wetland soils.
- Elevated salinity stimulates nitrous oxide emissions from wetland soils.
- Combined urban pollution and sea salts suppress CO_2 and CH_4 but enhance N_2O fluxes.

Keywords: greenhouse gas emissions; tidal wetlands; carbon dioxide; methane; nitrous oxide; saltwater intrusion

ABSTRACT

Wetlands serve an important role in regulating greenhouse gases globally. Anthropogenic stressors including elevated nutrients and contaminants from urban pollution and elevated salinity from saltwater intrusion have the potential to alter greenhouse gas emissions, particularly in tidal wetlands that experience these stressors simultaneously. We designed a laboratory soil core experiment to disentangle the separate and combined effects of saltwater intrusion (elevated salinity versus elevated sulfate) and urban pollution (elevated nitrogen versus elevated copper) on carbon and nitrogen cycling in wetland soils. We collected 45 intact soil cores from a brackish tidal wetland and applied chemical treatments for seven weeks in the laboratory. We measured greenhouse gas soil-to-atmosphere fluxes (CO_2, CH_4, N_2O) and porewater chemistry weekly. Soils were harvested at the end of the experiment to analyze soil chemistry and microbial carbon and nitrogen processing rates. Compared to the freshwater control, we found that elevated copper and sulfate (without elevated salinity) decreased CH_4 fluxes. Treatments with elevated salinity, sulfate, or both also reduced CO_2 fluxes. Likewise, carbon mineralization at the end of the experiment was lower for all treatments with elevated copper, sulfate, or salinity. In contrast, elevated salinity enhanced N_2O fluxes but did not affect potential denitrification rates at the end of the experiment. Combined urban pollution and sea salt enrichment suppressed carbon gas fluxes but enhanced N_2O emissions. Decreased carbon gas fluxes offset increased N_2O fluxes such that the saltwater, urban, and combined treatments reduced the total global warming potential by 6.9×, 2.6×, and 3.6×, respectively, relative to the freshwater control. Thus our laboratory experiment suggests in the short-term, saltwater intrusion could reduce global warming potential of coastal wetland soils in Southern New England with typical levels of urban pollution. Future research that addresses the response of coastal wetland

ecosystems to combined effects of urban pollution and saltwater intrusion will be critical for predicting long-term coastal wetland function under future land use and climatic conditions.

1. INTRODUCTION

Coastal wetlands are susceptible to effects of land development and climate change (IPCC, 2014; Small and Nicholls, 2003). Coastal population density has steadily increased in recent decades and is projected to rise across the globe (Crossland et al., 2005; Eurostat, 2010; Wilson and Fischetti, 2010). Land development associated with increasing human populations directly reduces wetland habitat and degrades wetlands by increasing nutrient and metal loads (Bergback et al., 2001; Carpenter et al., 1998; Davidson et al., 2010). Saltwater intrusion associated with sea level rise, droughts, storm surges, and other factors can affect wetland ecosystems by increasing ionic strength and sulfate concentrations (Herbert et al., 2015; Nicholls and Cazenave, 2010; Werner et al., 2013). Thus, coastal wetlands receive pollution from upstream developed land and saltwater intrusion from downstream coastal systems, potentially creating biogeochemical regime shifts that may drive changes in ecosystem function and associated greenhouse gas emissions (Helton et al., 2014). In this study, we evaluate the potential individual and combined effects of urban pollution and saltwater intrusion on carbon and nitrogen cycling in coastal wetland soils.

Elevated nitrogen loads from developed land can alter carbon and nitrogen processing in wetlands. Increased nitrogen loading is commonly associated with greater rates of denitrification (Wallenstein et al., 2016) and increased nitrous oxide (N_2O) emissions (Hefting et al., 2003; Liu and Greaver, 2009; Moseman-Valtierra et al., 2011). Nitrogen additions may have variable effects on carbon gas emissions. Nitrogen enrichment has been associated with both insignificant

effects on carbon dioxide (CO_2) and methane (CH_4) fluxes (Keller et al., 2005; Min et al., 2011; Moseman-Valtierra et al., 2011) and increased CH_4 fluxes (Cheng-Fang et al., 2012; Liu and Greaver, 2009) across a variety of ecosystems. Elevated nitrogen concentration may suppress methanotrophy, decreasing CH_4 uptake (Liu and Greaver, 2009).

Urban pollution contributes to metal enrichment of copper, cadmium, lead, and zinc above natural levels (Bebianno et al., 2015; Martínez-Santos et al., 2015; Yang et al., 2013). Many microbial processes, including denitrification and CH_4 oxidation, require metals in enzymes (Giller et al., 1998; Glass and Orphan, 2012). We found a positive correlation between copper and potential denitrification rates in a field survey that included the focal wetland in this study (Doroski Unpublished Data). However, metals are generally considered toxic at higher levels (Özbelge et al., 2007) and are associated with chronic toxicity in estuarine sediments (Cruz et al., 2014; Ochoa-Herrera et al., 2009). Other laboratory studies have found elevated copper had insignificant or inhibitory effects on denitrification (Magalhães et al., 2007). Additionally, lower rates of carbon mineralization were associated with increased copper concentrations (Luke et al., 2015; Nwuche and Ugoji, 2008); however, threshold toxicity of copper to biological systems can range orders of magnitude (Özbelge et al., 2007).

Research on the effects of saltwater intrusion on carbon and nitrogen cycling in wetlands has produced variable results (Herbert et al., 2015; Luo et al., 2017; Zhou et al., 2017). Microbial carbon and nitrogen pathways may be affected by increased salinity, sulfate, or both. Increased sulfate concentrations may increase sulfate reduction rates and associated carbon mineralization and CO_2 production (Chambers et al., 2011; Marton et al., 2012), but at the same time decrease CH_4 production since sulfate reducers should outcompete methanogens for available organic carbon (Helton et al., 2014; Neubauer et al., 2013). On the other hand, increased ionic strength or

sulfide toxicity may directly inhibit microbial processes, suppressing carbon mineralization (Luo et al., 2017). Carbon mineralization may also decrease with long-term sea salt enrichment though changes in carbon quality and availability (Neubauer et al., 2013).

There is still uncertainty of the dominant mechanisms driving changes in nitrogen cycling from increasing salinity (Zhou et al., 2017). Elevated ammonium release through cation exchange (Ardón et al., 2013) may alter the availability of ammonium for nitrifiers. Sulfide toxicity may directly affect nitrifiying and denitrifying bacteria, decreasing rates of nitrification, denitrification, and linked nitrification-denitrification (Joye and Hollibaugh, 1995). Alternatively, elevated sulfide may act as an electron acceptor for denitrification (Burgin and Hamilton, 2007). Individual studies have reported decreased (Craft et al., 2009; Marks et al., 2016; Rysgaard et al., 1999; Seo et al., 2008), insignificant (Magalhaes et al., 2005), and increased (Marks et al., 2016) denitrification rates with elevated salinity. In a recent meta-analysis, there was no significant effect of elevated salinity on denitrification rates and elevated salinity was associated with higher N_2O emissions in freshwater wetlands, lower N_2O emissions from salt marshes, and variable responses in brackish marshes (Zhou et al., 2017).

Recent research has begun to parse the effects of elevated salinity and sulfate (Chambers et al., 2011; Helton et al., In Press; Marton et al., 2012; Seo et al., 2008) or the effects of nutrient pollution and saltwater intrusion (Helton et al., 2014; Hu et al., 2016), but research is still needed to understand the complexity of responses when wetlands experience both saltwater intrusion and nutrient or metal pollution. Therefore, our objective was to disentangle the effects of urban pollution (elevated nitrate and copper) and saltwater intrusion (elevated salinity and sulfate) on carbon and nitrogen cycling in coastal wetland soils. We conducted an experiment in which intact soil cores received chemical treatments for seven weeks to simulate the combined and

individual effects of seawater (elevated salinity, sulfate, and salinity and sulfate) and urban pollution (elevated nitrate, copper, and nitrate and copper). We measured greenhouse gas (CO_2, CH_4, N_2O) fluxes weekly and potential denitrification and carbon mineralization rates at the end of the experiment.

2. MATERIALS AND METHODS

2.1 Soil Core Collection

On July 11, 2016, we collected 45 intact soil cores in PVC pipe (7.62 cm dia, 25 cm depth) from a tidal wetland dominated by a monotypic stand of *Typha spp.* located in Lord Cove of Lyme, CT U.S.A (41.365401, -72.367233). This wetland is at the forefront of saltwater intrusion as it is within the saltwater limit along the Connecticut River (CT DEEP, 1995). Soil samples were collected at low tide along the unvegetated bank of the channel where soils remain saturated even during low tide. We recorded surface water salinity and temperature with a handheld meter (model 556 MPS, YSI Inc., Yellow Springs, OH). At time of sampling, surface water salinity was 4.09 ppt, although during previous visits surface water salinity ranged from 0.22 to 0.87 ppt. Severe drought conditions from March to July in 2015 and 2016 may have induced saltwater intrusion by the July 2016 sampling date (NOAA National Centers for Environmental Information, 2016). Soils taken from a survey of 32 wetlands on the Connecticut coast in the summer of 2015 suggest soil copper and nitrate at the site were lower than surrounding wetlands. At our site soil copper was 9.4 mg kg dry soil $^{-1}$ (across all 32 sites, mean ± std error; 61 ± 10.4) and soil KCl extractable nitrate was 1.1 mg N kg dry soil $^{-1}$ (3.3 ± 0.41) (Doroski Unpublished Data).

2.2 Experimental Design

Soil cores were taken to the laboratory for an experiment in which eight treatments were applied for seven weeks (Table 1). Levels of salinity, nitrate, and copper were chosen based on environmentally relevant concentrations (Table 1). Artificial seawater (ASW) and artificial freshwater recipes (AFW) were used for treatments and as matrices for combination treatments (Table 1). The seawater treatment (ASW, 18 ppt) represented the upper threshold of the mesohaline salinity class (Cowardin et al., 1979). The saltwater treatment (ASW without sulfate,18 ppt) was made without sulfate to identify effects of salinity alone. The sulfate treatment was prepared with sulfate concentrations equivalent to those found in the seawater treatment but without elevated salinity to identify effects of sulfate without elevated salinity. The nitrate treatment was prepared to 2 mg N L^{-1} to represent nitrate river export and storm water concentrations (Mullaney, 2016). The copper treatment was prepared to 100 µg Cu L^{-1} to represent storm water concentrations (Odnevall Wallinder et al., 2009). All treatments included 4 mg L^{-1} of carbon as potassium acetate to provide carbon that would normally be supplied by river export.

After collection, the 45 intact soil cores were immediately sealed with a bottom cap. For 40 soil cores, we installed a drainage pipe fitted with a cloth filter at 15 cm soil depth and a lysimeter (simpler panel-mount micro sampler, SoilMoisture Equipment Corp., Goleta, CA) at 5 cm soil depth for soil porewater extraction. During installation and equilibration, we applied equal amounts of AFW as needed to keep soil cores inundated. After two weeks of equilibration, we harvested five soil cores to analyze initial soil characteristics. We randomly divided the remaining 40 cores into eight treatment groups, with five soil cores per treatment. Equal volumes (30 mL) of treatment were added to the cores three times a week for seven weeks. Soil cores

were kept inundated with approximately 2.5 cm of overlying water. To maintain a consistent 2.5 cm water depth, we drained 30 mL from each core through the 15 cm drainage pipe on eleven days over the course of the experiment.

We collected 10 ml of soil porewater prior to treatment (Week 0), 3 days after initial treatment (Day 3), and weekly (Weeks 1-7) for a total of nine soil porewater sampling dates. We measured soil-to-atmosphere greenhouse gas fluxes prior to treatment (from three cores of each treatment) and weekly after initial treatment for seven weeks (for all five cores in each treatment) for a total of eight greenhouse gas sampling dates (Weeks 0-7). At the end of the experiment we harvested, sieved (2 mm), and homogenized soils. Because we added chemical treatments to the top of soil cores we expected experimental effects to potentially decrease with soil depth as reactive solutes in treatments (e.g., sulfate, copper, and nitrate) were transformed; thus, surface soils were sectioned into 0-5 cm (upper) and 5-10 cm (lower) depths for analysis.

2.3 Greenhouse Gas Fluxes

During sampling, we created an air tight chamber by placing a PVC end cap greased with petroleum jelly and fitted with a gas sampling port on each soil core. Headspace samples were collected from each core over three 20-minute intervals by injecting 22 mL of CO_2-free air into the headspace, pumping the syringe three times to mix the gas, and extracting 22 mL of sample. Samples were injected into pre-evacuated glass vials which were loaded into a TurboMatrix 40 Trap Headspace Autosampler (PerkinElmer, Shelton, CT) for delivery to a Clarus 580 gas chromatograph with a flame ionization detector (FID) and methanizer to measure CO_2 and CH_4 and an electron capture detector (ECD) to measure N_2O.

Gas concentrations were corrected for CO_2-free air dilution and we converted measured gas concentrations (ppmv) to mg m^{-3} using the ideal gas law and measurements of barometric pressure and air temperature taken at the time of gas flux measurements (Holland et al., 1999). Soil-to-atmosphere gas flux was calculated as the slope of gas concentration versus time multiplied by the volume of the headspace on the sampling date (309 ± 30 ml) and divided by the soil core surface area (mg m^{-2} h^{-1}). We calculated the minimum detectable concentration difference (MDCD; Yates et al., 2006) for CO_2 and CH_4 with pairs of standards and for N_2O with pairs of ambient air samples. If the change in concentration over the full incubation was less than MDCD, we set the flux to zero. For changes greater than MDCD, we used the slope of the regression between gas concentration and time over the full incubation. We excluded fluxes from further analysis with poor linear relationships (i.e., r^2 <0.75).

2.4 Soil and Porewater Chemistry

Soils collected before treatments (n = 5) and soils harvested at the end of the experiment (n = 40) were dried at 105°C for 72 hours to determine moisture content and combusted at 550°C for four hours to determine soil organic matter (SOM) by the loss on ignition method (adapted from USDA-NRCS 1996). We extracted soil ammonium and nitrate with 2N KCl by adding 25 ml of KCl solution to 2.5 grams of field moist soils, shaking for 30 minutes at 200 rpm, centrifuging for 5 minutes at 2400 rpm, and filtering supernatant through Whatman 589/1 filters (adapted from Keeney and Nelson 1982). Porewater and KCl extractable ammonium concentrations were determined by the phenate method (APHA 1999) on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT). Porewater nitrate was analyzed on a Dionex Ion Chromatography System (ICS)-1100 (Thermo Fisher Scientific,

Waltham, MA). KCl extractable nitrate was analyzed by colorimetric determination of nitrate plus nitrite by enzymatic reduction (Campbell et al., 1997; Patton and Kryskalla, 2011) on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT).

We extracted soil chloride and sulfate by adding 25 ml of distilled, deionized water to 2.5 g of field moist soil, shaking for 30 minutes at 200 rpm, centrifuging for 5 minutes at 2400 rpm, and filtering supernatant through Whatman GF/F filters. Water extractable and porewater chloride and sulfate concentrations were analyzed on the ICS-1100 (Thermo Fisher Scientific, Waltham, MA). Porewater metal concentrations were analyzed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7700x with He collision cell, Agilent, Delaware, USA). All analyses were conducted at the University of Connecticut.

2.5 Carbon and Nitrogen Processing Rates

We measured denitrification enzyme activity on homogenized soils collected at the end of the experiment (Groffman et al., 1999). Denitrification enzyme activity is a measure of potential denitrification since the assays remove limiting factors; assays are conducted under anoxic conditions with excess carbon and nitrate. Following the procedure in Groffman et al (1999), we added 5 grams of field moist soil to 10 ml of DEA media (0.72 g KNO3, 0.5g glucose, 0.125g Chloramphenicol per liter of distilled, deionized water) in 125 ml glass flasks with gas-tight seals and flushed the headspace with N_2 gas to create anoxic conditions. We added 10 ml of acetylene gas to block the reduction of N_2O to N_2 gas. Headspace gas was sampled for four time-points (i.e. approximately 0, 30, 60, and 90 minutes after start of incubation) and analyzed for N_2O and MDCD according to section *2.3* above. Potential denitrification was calculated as the linear rate of evolved N_2O-N over time per gram of dry soil.

We measured carbon mineralization rates of 5 g of field moist soils in 100 mL sealed serum bottles over a three-day incubation. Headspace CO_2 was sampled immediately after bottles were sealed and after one and three days. We used the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978; West and Sparling, 1986) as an index for potential microbial activity. Substrate induced respiration was measured after adding 10 mL of yeast solution to 5 g of field moist soil in 40 mL sealed amber vials. Headspace CO_2 was sampled immediately after vials were sealed and after 2 and 4 hours. Headspace CO_2 samples for carbon mineralization and SIR were immediately injected into a LI-840A CO_2/H_2O Gas Analyzer (LI-COR, Lincoln, NE), and rates were calculated as the linear rate of evolved CO_2-C over time per gram of dry soil.

2.6 Data Analysis

We used one-way analysis of variance (ANOVA) (R function 'aov()') to compare soil nutrient and salt concentrations, cumulative greenhouse gas fluxes, and microbial processing rates among treatments. We calculated cumulative gas fluxes by linearly interpolating daily gas fluxes between sampling dates (Weeks 1-7) and then summing daily fluxes over the course of the experiment. To compare greenhouse gas fluxes (Weeks 1-7) and porewater chemistry (Weeks 0-7) between treatments over time, we used repeated measures analysis with a linear mixed effects model (R function 'lme()') (Pinheiro et al., 2016) and ANOVA (R function 'anova()'). When main effects of ANOVA were significant, post-hoc Tukey tests were conducted with the lsmeans package (Lenth, 2016) to identify differences among main effects. Normality was tested with the Shapiro-Wilk Normality Test, and parameters were square root or natural log (ln) transformed to improve normality as indicated in Section 3. Statistical analyses were performed using R version

3.3.2 (R Core Team 2016). We also converted cumulative CH_4 and N_2O fluxes to CO_2 equivalents by multiplying CH_4 by 28 and N_2O by 265, based on the IPCC's 100-year time horizon estimates (Myhre et al., 2013).

3 **RESULTS**

3.1 Porewater Chemistry

As intended, porewater chloride and sulfate concentrations for treatments with added salt and sulfate increased compared to treatments without added salt or sulfate, respectively (Figure 1a-b; Table 2; post-hoc Tukey HSD tests $p < 0.05$ for weeks 2-7). Porewater nitrate was typically below detection. Although our target concentration for porewater nitrate was 2 mg L^{-1}, for Week 0, 80% of porewater nitrate was below detection (detection limit = 0.021 mg L^{-1}). For the following sampling weeks, all of the samples were below detection (detection limits ranged from 0.032 to 0.17 mg L^{-1}). Porewater copper was also often below detection (detection limit = 2 µg L^{-1}) and not significantly different across treatments (Figure 1c, Table 2), although the highest porewater copper concentrations were measured in the copper treatment.

The elevated salinity of saltwater, seawater, and combined treatments mobilized higher concentrations of ammonium into porewater. Porewater ammonium concentrations generally peaked for treatments with elevated salinity (saltwater, seawater, and combined treatments) between Week 2 and Week 4 and then declined (Figure 1d). On Week 2, treatments with elevated salinity had significantly higher porewater ammonium compared to the freshwater control (post-hoc Tukey HSD tests, $p< 0.05$). By the last porewater sampling period (Week 7) the freshwater control did not significantly differ in porewater ammonium concentrations from

277 treatments with elevated salinity. Soil extractable solutes measured at the end of the experiment
278 exhibited similar patterns to porewater concentrations among treatments (Supplemental Table 1).
279
280 *3.2 Carbon Gas Flux and Mineralization*
281 Weekly CO_2 fluxes (Table 3; Figure 2a) and cumulative CO_2 fluxes (Figure 2b) varied
282 significantly by treatment, with elevated salinity and sulfate suppressing CO_2 emissions across
283 treatments. Weekly CO_2 fluxes for the saltwater, sulfate, and combined treatments tended to be
284 lower than for the freshwater control throughout the experiment and were significantly lower for
285 weeks three and five (post-hoc Tukey HSD tests $p<0.05$; Figure 2a). Fluxes of CO_2 from nitrate,
286 copper, and urban treatments were not significantly different than the freshwater control (post-
287 hoc Tukey HSD tests $p>0.05$; Figure 2a). Similarly, cumulative CO_2 fluxes over the course of the
288 experiment were significantly lower for sulfate and saltwater treatments and were lower (but not
289 significantly) for seawater, urban, and combined treatments relative to the freshwater control
290 (Figure 2b).
291 Relative to the freshwater control, fluxes of CH_4 tended to be higher for the nitrate
292 treatment and lower for the copper, sulfate, and seawater treatments (Figure 2c-d). Although
293 there were significant treatment and date effects for weekly CH_4 fluxes (Table 3), there were no
294 post-hoc significant differences among treatments by date (Tukey HSD, $p > 0.05$). For
295 cumulative CH_4 fluxes, the copper treatment was significantly lower than both the freshwater
296 control and nitrate treatments (Figure 2d). All other treatments (i.e., those with added sulfate,
297 elevated salinity, or added copper) tended to be lower than both the freshwater control and nitrate
298 treatment, but the differences were not significant (Figure 2d).
299 Carbon mineralization was significantly lower than the freshwater control for saltwater,

seawater, copper, and urban treatments for the upper soil section (Figure 3a). Substrate induced respiration shows similar patterns with treatments, although there were fewer statistical differences (Figure 3c). Carbon mineralization and SIR were generally lower in the lower soil section, with similar patterns to the upper soil section but with fewer significant differences among treatments, as expected (Figure 3b and d). Carbon mineralization and SIR were positively related to one another for the upper ($r^2 = 0.35$; $p = 0.0002$) and lower ($r^2 = 0.41$; $p < 0.0001$) soil sections.

3.3 Nitrous Oxide Flux and Potential Denitrification

Weekly N_2O fluxes from saltwater and seawater treatments were variable, but were generally higher compared to the freshwater control (Figure 2c; Table 3). There was a significant treatment effect for N_2O flux, but date and the interaction of date and treatment were not significant (Table 3). There were no post-hoc significant differences among treatments by date (Tukey HSD, $p > 0.05$). However, cumulative fluxes summed over the experiment show a clear response to salinity – all treatments with elevated salinity (saltwater, seawater, and combined) had significantly higher N_2O fluxes than the freshwater control (Figure 3f). Treatments with added nitrate and copper (nitrate, copper, and urban) did not have significantly different N_2O flux compared to the freshwater control (Figure 2f). Potential denitrification measured in soils harvested at the end of the experiment were typically higher in the upper soil section as expected, but not significantly different among any of the treatments for either soil depth (Figure 3e and f).

3.4 Global Warming Potential

Decreases in CO_2 and CH_4 in saltwater and copper treatments were enough to offset increases in N_2O in treatments with elevated salinity in terms of total global warming potential (Figure 4). Patterns in global warming potential were primarily driven by changes in CH_4 fluxes. The nitrate treatment was the only treatment that had global warming potential higher than the freshwater control (Figure 4), although the variability of the response of CH_4 flux to added nitrate was high (Figure 2d). The copper treatment was the only treatment with a negative global warming potential, driven by the strong decrease in CH_4 fluxes with added copper. The saltwater, urban, and combined treatments all reduced the total global warming potential relative to the freshwater control by 6.9×, 2.6×, and 3.6×, respectively.

4. **DISCUSSION**

4.1 Effects of Saltwater Intrusion

Our results suggest that both elevated salinity and sulfate decrease carbon mineralization and associated CO_2 and CH_4 fluxes from flooded wetland soils. Elevated salinity may directly affect soil microbial communities, reducing microbial biomass and respiration rates (Rath and Rousk, 2015). Microbes must divert energy from growth and survival to osmoregulation when external salt concentrations are high (Rath and Rousk, 2015; Schimel et al., 2007), potentially suppressing rates of carbon cycling. Similar to our experiment, previous short-term experiments in coastal wetlands have also found either decreased or no significant change in CO_2 and CH_4 emissions when salinity is increased with sodium chloride instead of sea salts (Chambers et al., 2011; van Dijk et al., 2015).

With saltwater intrusion, the expectation is that increased rates of sulfate reduction associated with elevated sulfate in sea salts will increase rates of overall carbon mineralization and CO_2 emissions from wetland soils (Chambers et al., 2011; Herbert et al., 2015). However, when we added sulfate alone we found the opposite pattern – sulfate enrichment significantly suppressed CO_2 emissions and carbon mineralization. When seawater (with sulfate) is used to increase salinity in coastal wetland experiments, CO_2 emissions typically increase although some variable responses have been observed (as reviewed by Luo et al., 2017). A decrease in CO_2 emissions with elevated sulfate could be because sulfide may be toxic to some microbes (e.g., Joye and Hollibaugh, 1995), potentially suppressing microbial respiration overall. Alternatively, sulfide may be used as an energetically favorable electron donor instead of organic carbon (e.g., Brunet and Garcia-Gil, 1996; Burgin et al., 2012), potentially decreasing the mineralization of organic carbon and production of CO_2. Understanding why the CO_2 response varies not only in magnitude but also in direction is important for projecting the carbon balance of wetlands under future sea level rise scenarios.

During saltwater intrusion, increased rates of sulfate reduction should suppress rates of methanogenesis and associated CH_4 production since sulfate reduction is more energetically favorable than methanogenesis (Megonigal et al., 2004). Prior research shows that elevated sea salts typically decrease CH_4 fluxes across a range of wetlands (as reviewed by Luo et al., 2017 but see Ardon et al., 2018; Helton et al., In Press; Weston et al., 2011), and CH_4 fluxes decline strongly with increasing sulfate concentrations in flooded soils (e.g., Helton et al., In Press). Our results here show a similar pattern with lower CH_4 fluxes from the treatment with added sulfate. This finding increases the body of research that supports the consistent finding of lower CH_4 emissions with increasing sulfate in flooded soils, which is particularly important in light of the

366 recent emphasis on broad scale tidal restoration to reduce CH_4 and offset greenhouse gas
367 emissions (Kroeger et al., 2017).

368 In contrast to CH_4 our results suggest that saltwater intrusion will stimulate N_2O
369 emissions from flooded wetland soils, driven by elevated salinity rather than increasing sulfate
370 concentrations. Several microbially-mediated N pathways that produce N_2O may be altered by
371 increasing salinity, including nitrification, denitrification, and dissimilatory nitrate reduction to
372 ammonium (DNRA); however, the response of these pathways is inconsistent (as reviewed by
373 Zhou et al., 2017). The increase may be because of preferential salt stress to the N_2O reductase
374 enzyme (Brunet and Garcia-Gil, 1996) or because of changing availability of nitrogen with
375 increased mobilization (Ardón et al., 2013). In our study, porewater ammonium was positively
376 related to N_2O flux ($r^2 = 0.20$; $p < 0.05$). Salt induced displacement of ammonium to porewater
377 potentially increases nitrogen available for nitrification and coupled nitrification-denitrification,
378 consequently increasing N_2O emissions with saltwater intrusion. Thus, as we consider restoring
379 tidal wetlands for their blue carbon potential (Kroeger et al., 2017) we need to understand the
380 trade off in global warming potential of increased N_2O across broad scales.

381 Even though there were clear effects of elevated salinity on net N_2O fluxes, potential
382 denitrification was not significantly different among treatments at the end of the experiment. As
383 expected, potential denitrification rates were higher in the shallowest soils; denitrification is a
384 high energy-yielding reaction, and redox reactions tend to proceed in order of their energy yield
385 with depth of the soil profile, particularly in wetlands where redox gradients are steep
386 (Schlesinger and Bernhardt, 2013). The lack of significance among treatments, specifically for
387 the shallow soil section where potential denitrification rates were high, may be because potential
388 denitrification assays block the final reduction step of N_2O to N_2, which may be particularly

sensitive to elevated salinity (Brunet and Garcia-Gil, 1996). Alternatively, the net N_2O response could be largely driven by N_2O producing reactions other than denitrification, like nitrification, which may be a major driver of N_2O production in some coastal wetlands (Morse and Bernhardt 2013). Research that addresses alternate pathways of N_2O production in response to saltwater intrusion (e.g., Yang and Silver, 2016) will be important for understanding how saltwater intrusion may change net N_2O fluxes under future conditions.

4.2 Effects of Urban Pollution

Overall our results suggest that copper may have an inhibitory effect on carbon mineralization and associated CH_4 emissions. The largest effect of any treatment on a gas flux was the inhibition of CH_4 flux by elevated copper, reducing cumulative CH_4 fluxes by over 100%. Lower CH_4 flux can be explained by two pathways: decreased CH_4 production and enhanced CH_4 consumption. Sanchez et al. (1996) found high concentrations of copper decreased CH_4 production and the number of methanogens, with 50% inhibition for Cu levels <10 - 250 mg/L. High levels of Cu are associated with both decreased acetoclastic and decreased hydrogenotrophic methanogenesis (Karri et al., 2006). However, those effects were observed at much higher Cu concentrations than in our experiment and is typically observed in wetlands. More likely, lower CH_4 can be explained by increased consumption of CH_4 by methanotrophy. Methanotrophic enzymes utilize copper (Glass and Orphan, 2012) suggesting CH_4 fluxes may be decreased due to increased methanotrophy. In environments of abundant copper, methanotrophic metalloenzymes utilize copper to catalyze the first step of CH_4 oxidation. Methanotrophs oxidize CH_4 as an energy and carbon source (Glass and Orphan, 2012), and therefore increases in methanotrophy does not necessarily produce CO_2 in equal amounts. In the field, high rates of

412 methanotrophy are likely fueled by oxygen from rhizosphere oxidation and tidal flow regimes.
413 In our lab experiment without plants or tidal flow, oxic treatment water added three times per
414 week and shallow water depths (~2.5 cm) maintained by draining water through the soil core
415 eleven times over the course of the experiment likely provided oxygen through advection and
416 diffusion to supply methanotrophic activity.

417 Although not significant, CH_4 flux was higher from the nitrate treatment than the
418 freshwater control in our experiment. Nitrogen enrichment increased CH_4 emissions by 95% and
419 decreased methanotrophy by 38% on average across a range of ecosystems (Lui and Greaver
420 2009) but did not have a significant effect on CH_4 emissions from some wetland ecosystems
421 (e.g., Moseman-Valtierra et al. 2011). The mechanisms that drive interactions between CH_4
422 emissions and elevated N are not well understood, but methane oxidizing bacteria, ammonia
423 oxidizers, and Archaea all depend on similar enzymes (Bodelier and Steenbergh, 2014); thus
424 understanding when and where nitrogen enrichment is likely to increase CH_4 emissions may be
425 important for understanding the magnitude of CH_4 emissions from wetlands under various
426 pollution and sea level rise scenarios. Our results suggest that while nitrate enrichment may lead
427 to increased CH_4 emissions from flooded wetland soils, the addition of Cu alone or in
428 conjunction with nitrate may offset the effect on CH_4 emissions, with Cu suppressing and
429 nitrogen enhancing CH_4 emissions.

430 Unlike carbon cycling, our urban pollution treatments did not have a significant effect on
431 potential denitrification or N_2O fluxes. This was in contrast to field patterns that suggested a
432 positive relationship between potential denitrification and copper concentration (Doroski
433 Unpublished Data). However, when we analyzed the relationship between potential
434 denitrification measured at the end of the experiment and average porewater copper

concentration, we found that potential denitrification rates, in the upper soils where denitrification rates tended to be higher, were positively related to copper when copper concentrations were relatively low (< 2 µg L^{-1}; $r^2 = 0.50$; $p < 0.05$, n = 13, for freshwater, copper, and urban treatments), but the relationship was insignificant over the full range of copper concentrations and for the lower soils where denitrification rates tended to be low. This suggest a potential stimulating effect of copper on denitrification at low concentrations, but an inhibitory effect at higher concentrations. Thus, the mechanisms driving the observed relationship between denitrification and copper may shift from use of copper in enzymes (Glass and Orphan, 2012) to toxicity (Özbelge et al., 2007) as copper availability increases.

We did not see increased N_2O emissions with added nitrate even though nitrogen enrichment is associated with enhanced N_2O fluxes for a variety of ecosystems (Liu and Greaver, 2009), including coastal wetlands where nitrogen enrichment may change wetlands from N_2O sinks to sources (e.g., Chmura et al., 2016). In our experiment, we chose to use average nitrate concentrations from river and storm water (i.e., 2 mg N L^{-1} equivalent to 0.53 mg m^{-2} per month), orders of magnitude less than is typically used in wetland fertilization experiments (e.g. 3 to 30 g N m^{-2} per month, Nelson and Zavaleta, 2012; Kearns et al., 2015). Thus, our experiment suggests that average nutrient concentrations received by wetlands in Southern New England likely are not high enough to substantially change N_2O emissions from wetlands in the short-term. However, N_2O in wetlands still likely responds to higher levels of nitrogen enrichment (e.g., downstream of agricultural fields, Helton et al., 2014).

4.3 Wetland Hydrology and Vegetation – Field versus laboratory conditions

Wetland vegetation and hydrologic dynamics play major roles in regulating soil biogeochemical transformations in field settings and over longer time horizons, which we did not address in our experimental design. Fluctuating water levels drive dynamic redox conditions that may alter or exacerbate the expected responses to elevated solutes in wetlands. For example, the response of microbes to elevated salinity can be exacerbated by low soil moisture (Rath et al., 2017); thus, there may be stronger salt-induced suppression of microbial respiration in variably flooded soils. Denitrification and nitrification which both produce N_2O occur under contrasting redox conditions, and denitrification rates may be highest in areas of fluctuating water levels due to coupled nitrification-denitrification (Hernandez and Mitsch, 2007). Thus, any differential response of a chemical stressor to denitrifying versus nitrifying bacteria may be important for determining the N_2O emission response in more or less inundated areas.

Wetland plant traits and community composition can also influence greenhouse gas fluxes from wetland ecosystems (Hooper and Vitousek, 1997; Sutton-Grier and Megonigal, 2011). Tidal wetland plants 1) have air filled, porous tissues (Aerenchyma) that act as conduits of gases between the atmosphere and sediment, 2) oxidize their rhizopheres, which can create conditions that drive high rates of coupled nitrification-denitrification and methane oxidation. And, 3) provide carbon and nutrients to soil microbes through root exudates and plant litter (Tobias and Neubauer, 2009). Across a salinity gradient, plant species composition ranges from freshwater species to saltwater tolerant species, but increasing salinity for freshwater plants can lead to plant mortality (Crain et al., 2004) and shifts in community composition (Glenn et al., 1995). Any changes to wetland plant communities over longer time scales may feedback to greenhouse gas emissions, which is not accounted for in our laboratory experiments.

4.4 Saltwater Intrusion and Urban Pollution Combined – Global Warming Potential

Overall sea salt enrichment and urban pollution either suppressed both CO_2 and CH_4 emissions or enhanced N_2O emissions in our soil core experiments. Reductions in CO_2 and CH_4 emissions from wetland soils were driven by responses to sulfate, salinity, and copper whereas changes to N_2O were driven only by elevated salinity. Decreases in CO_2 and CH_4 were enough to mostly offset increases in N_2O in terms of total global warming potential under saltwater intrusion, urban, and combined treatment conditions (Figure 4). Common chemicals in urban pollution had contrasting effects on greenhouse gas warming potential – elevated nitrate enhanced carbon gas emissions whereas copper suppressed carbon gas emissions, even shifting wetland soil cores from net sources to net sinks for greenhouse gases. This suggests that the magnitude of different chemicals in urban pollution could drive contrasting greenhouse gas responses in wetland soils, which is an important implication for future research to address at the ecosystem-scale. The direction and magnitude of these responses could determine whether urban pollution will compound or offset effects of sea salt enrichment from saltwater intrusion. In our soil core experiment, sea salt enrichment reduced global warming potential by 6.9×, whereas combined sea salt and urban pollution reduced global warming potential 3.6×. Previous work suggests that elevated nitrogen loads from agricultural loading can completely offset the global warming potential reduction of saltwater intrusion (Helton et al., 2014). Our work suggests that given common concentrations observed in southern New England wetlands, even in the face of increasing urban pollution, sea level rise could reduce rather than enhance global warming potential of coastal wetland soils in the short-term. As we continue to account for wetlands in global carbon and greenhouse gas budgets, further research is need to understand how dynamic

chemical environments may alter the greenhouse gas response to saltwater intrusion at the ecosystem-scale and over longer time horizons.

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FIGURE CAPTIONS

Figure 1. Average porewater concentrations (± standard error) of a) chloride (Cl^-), b) sulfate (SO_4^{2-}), c) copper (Cu), and d) ammonium (NH_4^+) over the seven-week experiment. Results from repeated measures ANOVA are reported in Table 2.

Figure 2. Weekly greenhouse gas fluxes (± standard error) for weeks three to seven of the experiment and cumulative greenhouse gas fluxes for weeks one to seven for a-b) carbon dioxide (CO_2), c-d) methane (CH_4), and e-f) nitrous oxide (N_2O). For weekly greenhouse gas fluxes, results from repeated measures ANOVA are reported in Table 3. For cumulative greenhouse gas fluxes, CO_2 and N_2O fluxes were square root transformed and CH_4 fluxes were natural log transformed before ANOVA, and letters denote significance ($p < 0.05$) from post-hoc Tukey HSD tests.

Figure 3. Average (± standard error) for the upper (0-5 cm) and lower (5-10 cm) soil depths for a-b) carbon mineralization, c-d) substrate induced respiration (SIR), and e-f) denitrification potential for soils harvested at the end of the experiment. Carbon mineralization, SIR, and denitrification potential were natural log transformed before ANOVA, and letters denote significance ($p < 0.05$) from post-hoc Tukey HSD tests.

Figure 4. Average global warming potential of cumulative greenhouse gases.

Table 1. Description of experimental treatments.
Artificial freshwater = AFW. Artificial seawater = ASW.

Treatment Group	Treatment Name	Components[1]	Salinity (ppt)
Freshwater control	Freshwater	AFW[2]	0.08
Marine salts	Seawater	ASW[3]	18
	Saltwater	ASW without sulfate[4]	18
	Sulfate	Sulfate to levels in ASW[5]	2
Urban contaminants	Nitrate	AFW + 2 mg L^{-1} N as $NaNO_3$	~0.08
	Copper	AFW + 100 ug L^{-1} Cu as $CuCl_2$	~0.08
	Urban	AFW + 2 mg L^{-1} N as $NaNO_3$ + 100 ug L^{-1} Cu as $CuCl_2$	~0.08
Marine salts + Urban contaminants	Combined	ASW + Nitrate + Copper	18

[1] All treatments received 4 mg C L^{-1} as potassium acetate; the average concentrations of total organic carbon in CT rivers (Mullaney, 2016)
[2] Prepared following (Smith et al., 2002)
[3] Prepared following (Kester et al., 1967)
[4] Prepared following (Kester et al., 1967) with K_2SO_4 replaced by KCl
[5] Prepared with K_2SO_4

Table 2. Results from repeated measures ANOVA for pore water sulfate (SO_4^{2-}), chloride (Cl^-), ammonium (NH_4^+), and copper (Cu) for Weeks 0-7.

	Cl^-			SO_4^{2-}			NH_4^+			Cu		
	df	F-value	P	*df*	F-value	P	*df*	F-value	P	*df*	F-value	P
Treatment	7	79.89	<0.0001	7	37.06	<0.0001	7	6.22	<0.0001	7	1.31	0.28
Date	8	19.98	<0.0001	8	51.66	<0.0001	8	32.94	<0.0001	8	46.4	<0.0001
Date*Treatment	56	5.67	<0.0001	56	11.51	<0.0001	56	2.71	<0.0001	56	2.41	<0.0001

Table 3 Results from repeated measures ANOVA for weekly greenhouse gas fluxes for Weeks 1-7. N_2O fluxes were natural log transformed before analysis.

	CO_2 Flux			CH_4 Flux			N_2O Flux		
	df	F-value	P	*df*	F-value	P	*df*	F-value	P
Treatment	7	4.69	0.001	7	2.60	0.03	7	4.92	0.0007
Date	6	14.8	<0.0001	6	2.29	0.04	6	1.64	0.14
Date*Treatment	42	2.99	<0.0001	42	1.38	0.08	42	0.61	0.97

Supplemental Table 1. Mean (standard deviation) of soil physiochemical properties including organic matter (SOM), extractable ammonium (NH_4^+-N), extractable chloride (Cl^-) and sulfate (SO_4^{2-}) prior (Ambient) and at the end of the experiment. ANOVAs were significant for each variable at each depth interval at $p<0.05$ and df = 7. F values are reported for each ANOVA. *Ambient soil cores were not included in ANOVAs. NH_4^+-N was square root transformed and Cl^- and SO_4^{2-} were ln transformed prior to analysis. Letters indicate significant differences among treatments ($p< 0.05$) from post-hoc Tukey HSD tests.

	Cl^- mg kg^{-1} soil		SO_4^{2-} mg kg^{-1} soil		NH_4^+-N mg kg^{-1} soil		SOM %	
	0-5 cm	5-10 cm	0-5 cm	5-10 cm	0-5 cm	5-10 cm	0-5 cm	5-10 cm
F-value	166.6	102.3	6.23	4.55	5.00	4.73	5.92	2.47
Ambient	4714* (1271)	4056* (1788)	1382* (420)	1017* (298)	142* (70.2)	106* (37.7)	17.2* (1.1)	16.6* (1.3)
Freshwater	1289^{c} (319)	1853^{cd} (278)	2462^{b} (531)	1985^{abc} (480)	72.8^{ab} (49.2)	113^{ab} (40.8)	14.8^{abc} (1.0)	14.9^{b} (1.0)
Sulfate	1117^{c} (206)	1377^{d} (275)	6263^{a} (1389)	3345^{a} (305)	18.2^{b} (15.3)	56.2^{c} (21.6)	16.4^{a} (1.4)	17.6^{a} (1.7)
Saltwater	19115^{a} (1791)	10678^{b} (1954)	1823^{b} (295)	1399^{c} (446)	35.3^{ab} (5.55)	75.1^{abc} (18.9)	16.4^{a} (1.3)	16.4^{ab} (1.4)
Seawater	11837^{b} (2975)	8334^{b} (2419)	2942^{b} (1272)	2003^{abc} (774)	29.5^{b} (12.4)	63.5^{bc} (20.6)	15.7^{ab} (0.6)	15.9^{ab} (1.3)
Nitrate	1059^{c} (238)	1848^{cd} (309)	2276^{b} (169)	1768^{bc} (363)	100^{a} (57.8)	136^{a} (27.3)	13.8^{bc} (1.4)	15.3^{ab} (1.0)
Copper	1444^{c} (429)	2270^{c} (540)	2469^{b} (401)	1732^{bc} (208)	61.2^{ab} (21.7)	105^{abc} (30.0)	13.5^{bc} (1.1)	14.8^{b} (1.3)
Urban	1302^{c} (135)	1634^{cd} (300)	2223^{b} (677)	2173^{abc} (628)	30.0^{b} (18.6)	73.6^{bc} (20.3)	13.9^{bc} (0.7)	14.9^{b} (1.5)
Combined	23993^{a} (8573)	17288^{a} (3787)	3418^{b} (1615)	2562^{ab} (688)	22.3^{b} (16.8)	70.8^{bc} (30.8)	15.1^{abc} (0.6)	16.3^{ab} (1.1)

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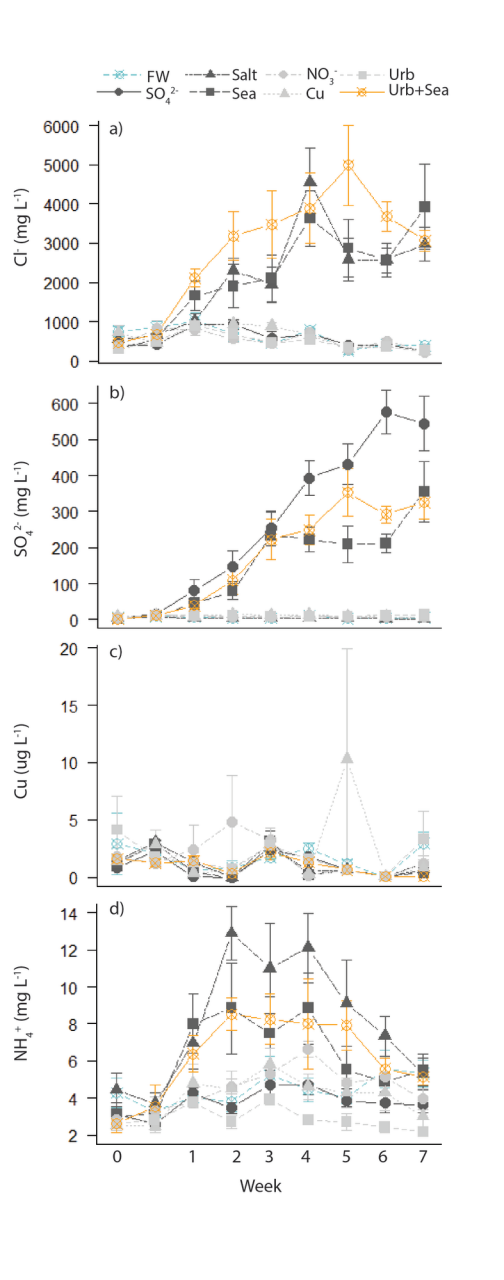
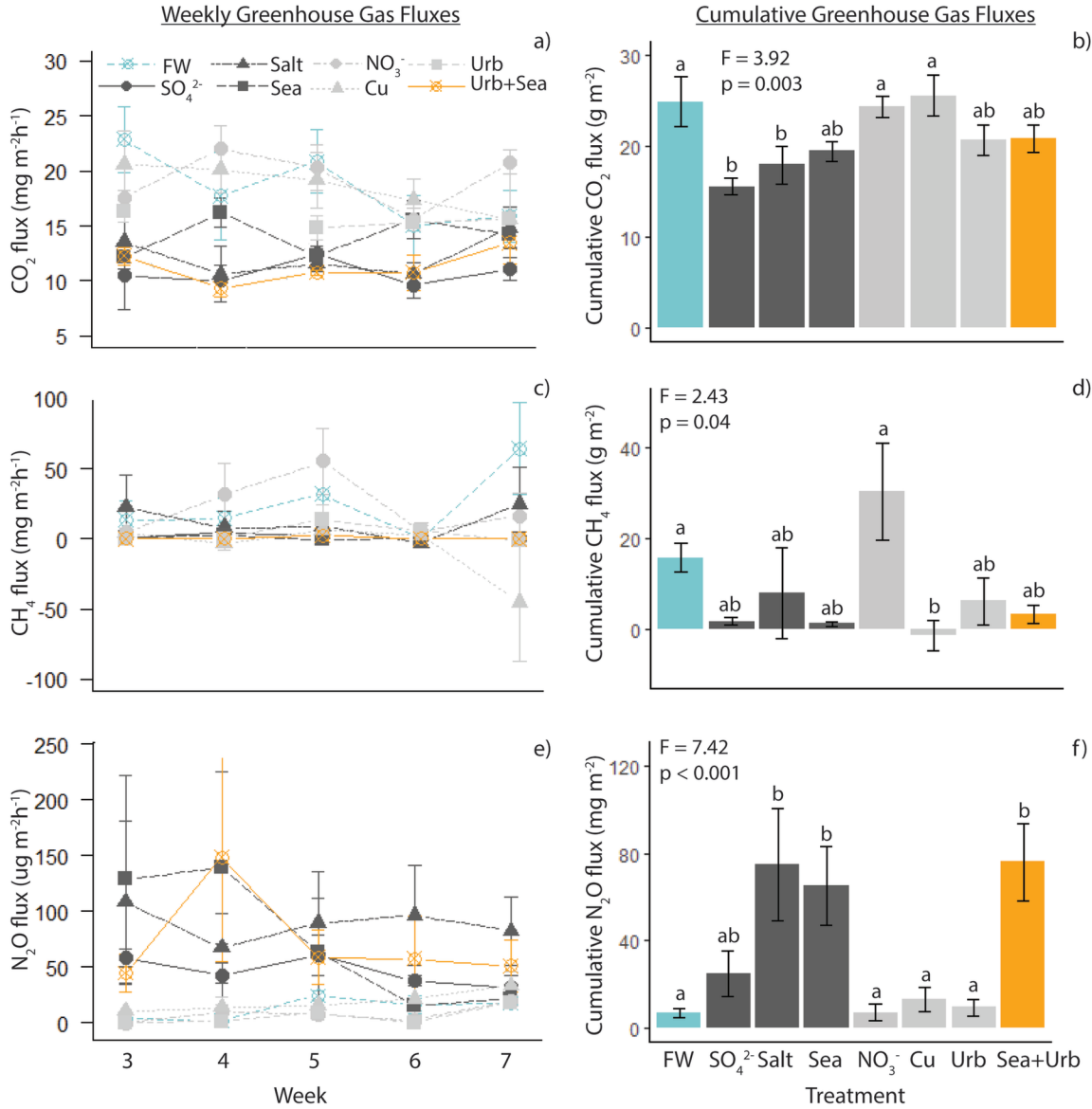
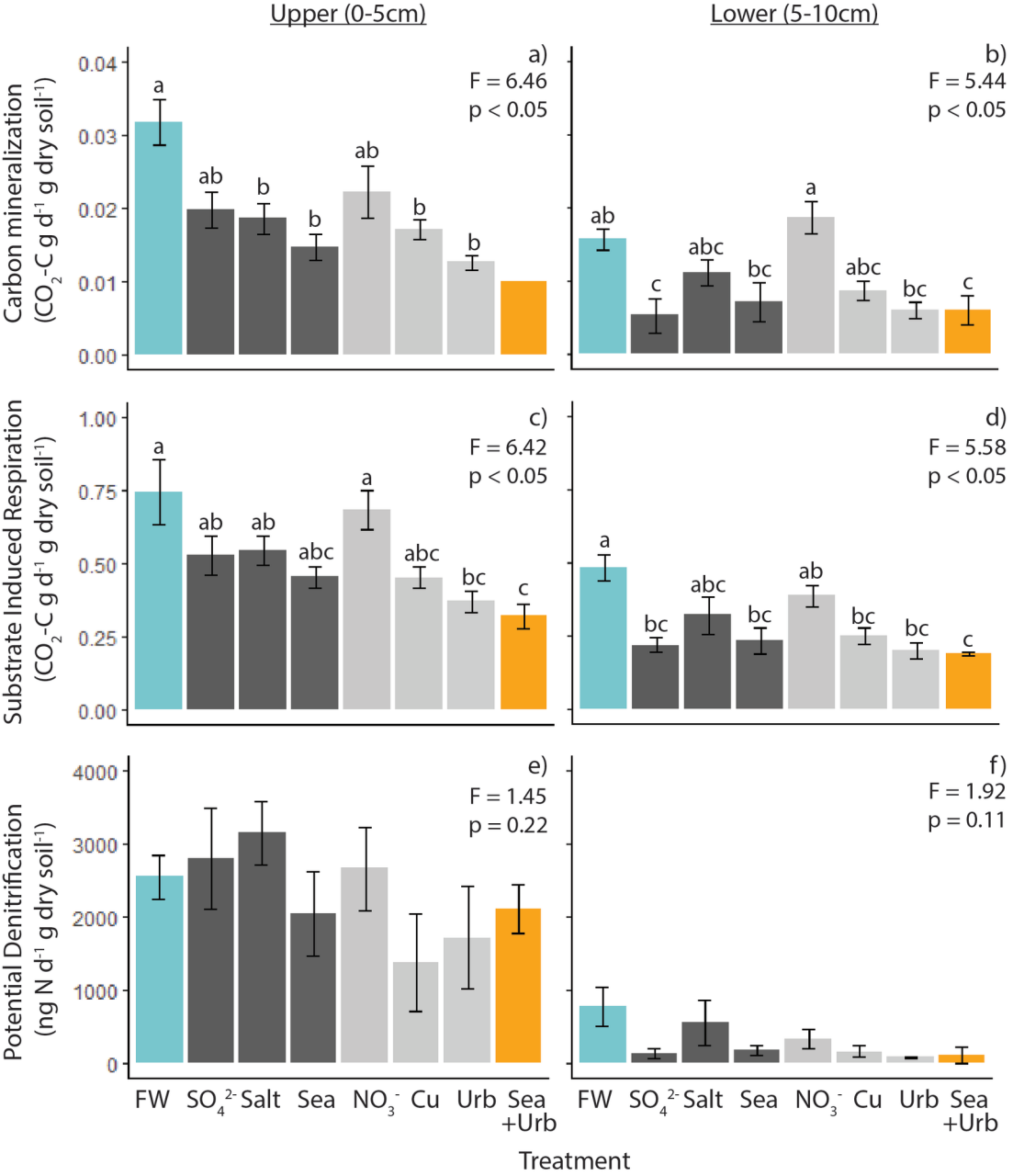
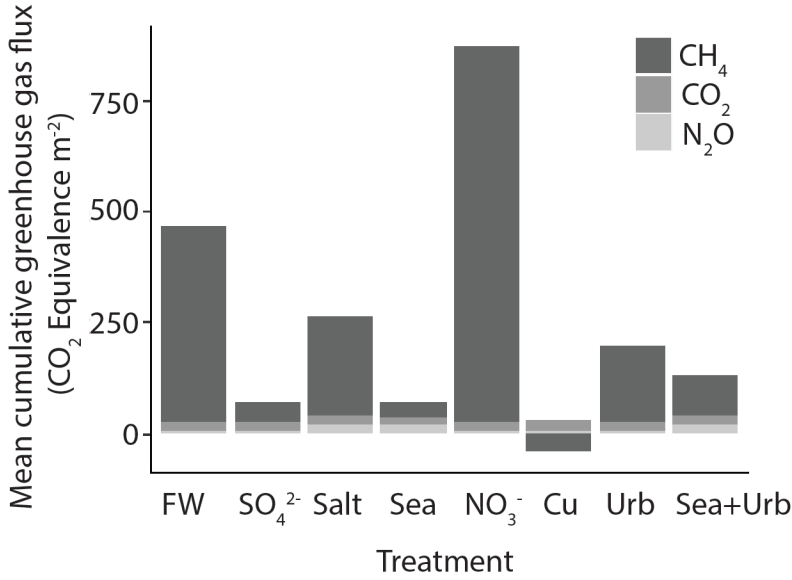

FW
Salt
NO3-
Urb
SO4 2-
Sea
Cu
Urb+Sea
a)
Cl- (mg L-1)
6000
5000
4000
3000
2000
1000
0
b)
SO4 2- (mg L-1)
600
500
400
300
200
100
0
c)
Cu (ug L-1)
20
15
10
5
0
d)
NH4+ (mg L-1)
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
Week


Weekly Greenhouse Gas Fluxes
Cumulative Greenhouse Gas Fluxes
a)
b)
c)
d)
e)
f)
FW
SO4 2-
Salt
Sea
NO3 -
Cu
Urb
Urb+Sea
CO2 flux (mg m-2h-1)
Cumulative CO2 flux (g m-2)
F = 3.92
p = 0.003
CH4 flux (mg m-2h-1)
Cumulative CH4 flux (g m-2)
F = 2.43
p = 0.04
N2O flux (ug m-2h-1)
Cumulative N2O flux (mg m-2)
F = 7.42
p < 0.001
Week
Treatment
FW
SO4 2-
Salt
Sea
NO3 -
Cu
Urb
Sea+Urb


Upper (0-5cm)
Lower (5-10cm)
a)
F = 6.46
p < 0.05
b)
F = 5.44
p < 0.05
c)
F = 6.42
p < 0.05
d)
F = 5.58
p < 0.05
e)
F = 1.45
p = 0.22
f)
F = 1.92
p = 0.11
Carbon mineralization (CO_2-C g d^{-1} g dry $soil^{-1}$)
Substrate Induced Respiration (CO_2-C g d^{-1} g dry $soil^{-1}$)
Potential Denitrification (ng N d^{-1} g dry $soil^{-1}$)
FW
SO_4^{2-}
Salt
Sea
NO_3^-
Cu
Urb
Sea +Urb
Treatment


Mean cumulative greenhouse gas flux
(CO_2 Equivalence m^{-2})
750
500
250
0
CH_4
CO_2
N_2O
FW
SO_4^{2-}
Salt
Sea
NO_3^-
Cu
Urb
Sea+Urb
Treatment