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Wetland Ecosystem Functions in a Changing Coastal Landscape: Effects of Urban Runoff, Saltwater Intrusion, and Restoration

April A. Doroski

B.S., The Pennsylvania State University, 2013

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APPROVAL PAGE

Master of Science Thesis

**Wetland Ecosystem Functions in a Changing Coastal Landscape:
Effects of Urban Runoff, Saltwater Intrusion, and Restoration**

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Chapter 1. The effects of salinity and metals on carbon and nitrogen processing in restored and unrestored coastal wetlands

1.1 ABSTRACT

The recovery of wetland function after tidal flow restoration (TFR) may be influenced by additional anthropogenic stressors including contaminant loading and sea level rise. Our objectives were to examine the effects of TFR on wetland biogeochemical functions and to identify the main biogeochemical drivers of nitrogen and carbon cycling (i.e. potential denitrification (DEA), carbon mineralization (C-Min), and microbial biomass) in restored and unrestored wetlands. Soil cores were collected in June 2015 from 32 saltwater, brackish, and freshwater tidal wetlands in coastal Connecticut, U.S.A. and analyzed at 0-5 cm (top layer) and 5-10 cm (bottom layer) depth intervals. We found lower DEA (mean \pm SEM) in restored (185 ± 43.3 ng N g⁻¹ soil hr⁻¹) compared to unrestored (903 ± 383 ng N g⁻¹ soil hr⁻¹) wetlands in the bottom layer ($p < 0.05$). In the top layer, DEA and microbial biomass increased with time since restoration ($p < 0.05$) suggesting restored wetlands have potential for functional recovery over time. Soil extractable ammonium and C-Min were greater in restored (101 ± 14.3 mg NH₄⁺ kg⁻¹; 0.182 ± 0.03 CO₂ C g day⁻¹ g⁻¹) compared to unrestored (61 ± 11.2 mg NH₄⁺ kg⁻¹; 0.108 ± 0.02 CO₂ C g day⁻¹ g⁻¹) wetlands in the top layer ($p < 0.05$; $p < 0.05$) which may be due to labile carbon availability from tidal flow. Salinity and lead (Pb) were negatively correlated with DEA ($p < 0.05$) and microbial biomass ($p < 0.05$), while manganese (Mn) and copper (Cu) were positively correlated with DEA ($p < 0.05$) and microbial biomass ($p < 0.05$). We found parameters associated with both runoff (i.e. Cu and Pb) and tidal flow (salinity) influenced nitrogen and carbon cycling.

1.2 INTRODUCTION

Wetland degradation reduces the capacity of wetlands to provide essential ecosystem services such as nutrient cycling, carbon sequestration, and water purification (Tiner 1989; Mitsch and Gosselink 2015). Wetlands, especially in coastal areas, are threatened by stressors associated with increasing coastal population density and development which has steadily risen since the 1960s (Wilson and Fischetti 2010). Development associated stressors include land conversion, runoff, contaminant loading, and altered hydrology (Crossland et al. 2005; Stedman and Dahl 2008). Additionally, coastal wetlands are susceptible to climate change risks associated with increased extreme weather events (i.e. storms and droughts) and sea level rise (IPCC 2014). In urban and agricultural areas, interactions between saltwater intrusion and runoff create complex biogeochemical regime shifts (Helton et al. 2014) that may alter wetland ecosystem function.

Saltwater intrusion elevates concentrations of ions (i.e. Cl^- , Na^+ , SO_4^{2-} , Mg^{2+}), which may have varying effects on microbial mediated nitrogen and carbon cycling in wetlands. In a laboratory experiment, Magalhaes et al. (2005) found no effects of salinity treatments (0, 15, 30 psu) on denitrification (the microbial reduction of nitrate to dinitrogen gas), suggesting presence of salt tolerant denitrifying species. In contrast, in field and laboratory experiments, elevated salinity levels caused a decrease in denitrification (Rysgaard et al. 1999; Putnam Duhon et al. 2012) potentially due to osmotic stress of microbes (Panswad and Anan 1999; Uygur 2006). Denitrification may also be inhibited or enhanced by elevated sulfate from seawater. Hydrogen sulfide produced by sulfate reduction inhibits the last step of denitrification to N_2 gas (Senga et al. 2006; Pan et al. 2013b). Sulfide may also serve as an alternate electron donor for denitrification (Burgin and Hamilton 2007), potentially increasing rates of denitrification.

Carbon cycling is influenced by saltwater intrusion by direct and indirect effects on microbial processing (Pathak and Rao 1998), plant communities (McKee and Mendelssohn 1989), and organic matter quality and quantity (Morrissey et al. 2014). The effects of salinity on carbon mineralization are still unclear (Herbert et al. 2015). After years of in-situ saltwater treatments, carbon mineralization rates (CO_2 and CH_4 production) were reduced compared to freshwater plots (Neubauer et al. 2013). This suggests sulfate reducers out-compete methanogens (Bartlett et al. 1987a; Megonigal et al. 2004). In contrast, Weston et al. (2011) found increased CO_2 and CH_4 production in wetland soil cores receiving saltwater intrusion treatments. Effects of saltwater intrusion on carbon cycling are further complicated by interactions with plant nutrient cycling and community shifts (Krauss et al. 2012). Excess salinity caused plant mortality and can lead to a community shift dominated by salt tolerant species (Glenn et al. 1995). Factors including the quality and quantity of autochthonous organic inputs of plants (Neubauer et al. 2013) and duration of saltwater intrusion influence the magnitude and response of carbon cycling (Weston et al. 2010; Marton et al. 2012; Neubauer et al. 2013).

High density urban development in coastal zones (Small and Nicholls 2003) further complicates the biogeochemical response of wetlands to saltwater intrusion by contributing excess nutrients (Carpenter et al. 1998) and urban contaminants such as Cu and Pb (Bergback et al. 2001; Revitt et al. 2014). Contaminants can accumulate over time in coastal wetlands (Williams et al. 1994), creating a potential legacy effect on carbon and nitrogen cycles. Carbon and nitrogen processing are linked with metals through enzymatic requirements (e.g., Cu for denitrification, (Glass and Orphan 2012)), redox pairs for microbial metabolism (Mn and Fe reduction (Lovley and Phillips 1988; Lovley 1991; Nealson and Myers 1992; Thamdrup 2000)),

and toxicity effects for enzymes or microbial metabolism (Giller et al. 1998; Rajapaksha et al. 2004).

In addition to chemical stressors, wetlands have undergone hydrologic stress due to drainage, impoundments, and installation of physical barriers such as roads, tidal gates, and undersized culverts. Tidal flow restoration (TFR) restores wetland hydrology to improve fish passage upstream, restore habitat, and reduce invasive species (Chambers et al. 2002; Elphick et al. 2015), but also increases salinity. Compared to aforementioned restoration goals, there is less focus on improving and monitoring biogeochemical functional post restoration. TFR exhibits faster biogeochemical recovery than other restoration types, however, recovery can take decades to over a century (Moreno-Mateos et al. 2012).

Therefore, the objectives of this study were to 1) determine the effects of TFR on carbon and nitrogen cycling in wetland soils and to 2) identify drivers of carbon and nitrogen cycling in wetland soils across gradients of salinity and metal contamination. The patterns of ecosystem function across a salinity gradient offers insight into how wetlands may respond to future saltwater intrusion. We hypothesized carbon and nitrogen cycling would be reduced in restored compared to unrestored wetlands and carbon and nitrogen cycling would be influenced by both salinity and urban contaminants across sites.

1.3 METHODS

1.3.1 Study Sites

In June 2015, we collected soil cores from a total of 32 tidal wetlands along the Long Island Sound in Connecticut (CT), U.S.A, including 17 restored tidal wetlands and 15 unrestored tidal wetlands (Fig. 1.1). Wetland sampling sites encompass freshwater, brackish, and saltwater sites with surface water salinity ranging from 0.03 to 29.04 ppt at time of sampling.

In order to understand effects of development intensity on wetland nutrient levels, metal concentrations, and microbial processing rates, we quantified developed land cover of the watershed draining into each sampling site (NHDPlus 2011). Developed land cover encompassed developed classes of the National Land Cover Database 2011 ranging from low-intensity (20% - 40% impervious surface) to high intensity development (80% - 100% impervious surface) (Homer et al. 2015). Sampling site watershed development intensity (> 20% impervious surface) ranged from less than 1% to 79% (mean \pm SEM, 20 ± 2.6 %) with mean watershed size of 3867 ± 1204 km².

TFR sites and unrestored sites were identified with help from CT DEEP (H. Yamalis and R. Wolfe, *personnel communication*, March 2, 2015). TFR sites were restored by one or more of the following techniques: culvert replacement, fill removal, installation of self-regulating tide gates, and tide gate removal. Completion of TFR ranged from 1-23 years prior to sampling. Unrestored wetland sites were chosen as tidal wetlands from the 1990s tidal wetlands shapefile (CT DEEP 1999) (with no known TFR) near restored sites, when possible.

1.3.2 Field Sampling and Soil Processing

At each site, three to four soil cores were collected with a slide hammer (5 cm dia, 15 cm length) within three hours of high tide to control for tidal influence. Samples were collected within ~500 meters of wetland edge and from areas of varying plant communities when possible. Additionally, we recorded surface water salinity and temperature with a handheld meter (model 556 MPS, YSI Inc., Yellow Springs, OH) and air temperature and barometric pressure with a handheld weather station (Kestrel Meters, Minneapolis, MN). Soil cores from each site were sectioned into 0-5 cm and 5-10 cm depths. For each depth, soil cores were composited, sieved (2

mm), and homogenized. Soil samples were analyzed at both depth intervals for soil chemical properties and carbon and nitrogen processing rates.

1.3.3 Soil Chemistry

Soil organic matter (SOM) was determined by loss on ignition (LOI). Samples were dried at 105°C to determine moisture content and combusted at 550°C to determine SOM (adapted from USDA-NRCS 1996).

Soil ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) were extracted with 2M KCl (soil:KCl = 1:10) (adapted from Keeney and Nelson 1982) and analyzed on a SmartChem@200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT). $\text{NH}_4^+\text{-N}$ was determined by the phenate method (APHA 1999) and $\text{NO}_3^-\text{-N}$ by colorimetric determination of $\text{NO}_3^-\text{-N}$ plus nitrite ($\text{NO}_2^-\text{-N}$) by enzymatic reduction (Campbell et al. 1997; Patton and Kryskalla 2011). Ninety-seven percent of samples analyzed for $\text{NO}_3^-\text{-N}$ were below the detection limit (0.11 mg L^{-1}). Therefore, $\text{NO}_3^-\text{-N}$ was not included in further analysis. All $\text{NH}_4^+\text{-N}$ concentrations were above the detection limit (0.12 mg L^{-1}).

Soil electrical conductivity (EC) was determined using the soil: water ratio, by volume, of 1:5 ($\text{EC}_{1:5\text{vol}}$) (USDA-NRCS 2011). EC measurements were made with an Oakton Con5 Acorn Series Conductivity/°C Meter (Oakton Instruments, Vernon Hills, IL). Additionally, water extractable (soil: water = 1:10) salt anions (i.e. chloride (Cl^-) and sulfate (SO_4^{2-})) were analyzed on a Dionex Ion Chromatography System (ICS)-1100 (Thermo Fisher Scientific, Waltham, MA). As expected, Cl^- and SO_4^{2-} concentrations were both positively correlated with soil EC (Cl^- $r^2=0.89$, SO_4^{2-} $r^2=0.78$, both $p < 0.001$) and were consequently excluded from further analysis. Soil EC was included in analysis as a surrogate of combined effects of both salt anions (i.e. Cl^- and SO_4^{2-}).

Total soil metal concentrations of redox active metals, Fe and Mn, and common urban metals, Cu and Pb, were determined by acid digestions with 70% HNO₃ (trace metal grade) and 30% H₂O₂ according to Method 3050B (US EPA 1996). Metals were analyzed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7700x with He collision cell, Agilent, Delaware, USA). We found a significant positive correlation between urban metals (Cu and Pb) and watershed development for the top ($p < 0.01$, Cu $r^2 = 0.22$, Pb $r^2 = 0.41$) and bottom ($p < 0.05$ Cu $r^2 = 0.17$, Pb $r^2 = 0.30$) soil layers and therefore used these two metals as indicators of developed land use and associated processes (i.e. runoff).

1.3.4 Soil Assays

We measured denitrification enzyme activity (DEA) with the acetylene block technique (Groffman et al. 1999), amended with excess carbon (glucose), nitrate (KNO₃), and chloramphenicol as an index of potential denitrification. We weighed five grams of soil into 125 mL Erlenmeyer flask, added 10mL of the carbon, nitrate, and chloramphenicol media, and sealed the flask with a Suba-Seal® septa. Conditions were made anoxic with N₂. Headspace N₂O was collected from the incubations at an initial time point (acetylene injected) and for 30 minute increments thereafter (N₂ injected) for four time points total (i.e. T0, T30, T60, T90). N₂O was analyzed with a Clarus 580 gas chromatograph (GC) equipped with an electron capture detector (ECD) (PerkinElmer, Shelton, CT). Samples were delivered to the GC with a TurboMatrix 40 Trap Headspace Autosampler (PerkinElmer, Shelton, CT).

We calculated the minimum detectable concentration difference (MDCD) for N₂O with the average difference between sample pairs ($\mu_{\text{pair diff}}$) of a gas sample standard and the standard deviation ($\sigma_{\text{pair diff}}$) with Eq. [1] (Yates et al. 2006). For fluxes above MDCD, when the $r^2 > 0.85$ we used the slope based on the full 90 minute incubation. When gas accumulation was non-linear

(i.e., $r^2 < 0.85$) over the full incubation, we calculated flux, excluding T90, from incubation period above MD CD and with $r^2 > 0.85$. If the incubation periods still yielded $r^2 < 0.85$, we calculated the flux as the slope of concentration between the first two time points.

$$MD CD = \mu_{pair\ diff} + (2\sigma_{pair\ diff}) \quad [1]$$

Potential denitrification rates were calculated as the linear rate of evolved N_2O -N over time per gram of dry soil.

Aerobic carbon mineralization (C-Min) rates were measured as CO_2 accumulation of field moist soils over a three day incubation. Five grams of soil were weighed from each sample into a 100 mL serum bottle and sealed with a septum and aluminum cap. Headspace samples were collected at an initial time point, and after one and three days. Headspace CO_2 was sampled by injecting five mL of CO_2 -free air into the serum bottle, mixing the headspace gas by pumping the syringe three times, and extracting five mL of sample. The sample was injected into a LI-840A CO_2/H_2O Gas Analyzer (LI-COR, Lincoln, NE) to measure CO_2 concentration.

We used the substrate induced respiration (SIR) method (Anderson and Domsch 1978; West and Sparling 1986) as an index for soil microbial biomass. SIR is positively correlated with total microbial biomass (Britam and Neely 1990) and is a commonly used as an indirect measurement of total microbial biomass (Bååth and Anderson 2003). Five grams of soil and 10 mL of yeast solution, delivering 20 mg yeast per gram of soil, were added to a 40 mL amber vial and sealed with a septum and cap. Headspace CO_2 was sampled similarly to C-Min but with a one mL injection over an initial time point, after two, and four hours. C-Min and SIR rates were calculated as the linear rate of accumulated CO_2 -C over time per gram of dry soil.

1.3.5 Statistical Analyses

Statistical analyses were performed using R version 3.1.3 (R Core Team 2015), with R Studio Interface version 0.98.1103 (R Studio Team 2014). Normality was tested with the Shapiro-Wilk Normality Test (R function 'shapiro.test()'). All parameters were natural log (ln+1) transformed to improve normality except for SOM in both layers and Fe in the bottom layer.

We used Student's t-test (R function 't.test()') to compare the differences of chemical properties and microbial processing rates between restored and unrestored wetlands for both the top and bottom soil layers. Simple linear regression (R function 'lm()') was used to determine relationships between year since restoration and soil properties, and between watershed development and soil metal content.

Additionally we performed multiple linear regression model selection by exhaustive search (R function 'regsubsets()') to examine the effects of soil EC (a measure of salinity), SOM, nutrients ($\text{NH}_4^+\text{-N}$), and metal concentrations (Fe, Mn, Cu, Pb) on DEA, C-Min, and microbial biomass. Results for DEA, C-Min, and microbial biomass combine restored and unrestored wetlands data and were analyzed by depth. The best fit model was selected by the lowest Akaike Information Criterion (AIC) (R function 'extractAIC()') given all combinations of parameters (Burnham et al. 2011).

1.4 RESULTS

1.4.1 Effects of Restoration

DEA was significantly lower in restored than unrestored wetlands (bottom layer, $p < 0.05$, $t = -2.48$, $df = 28.5$; Fig. 1.2a); however, DEA increased with time since restoration (top layer, $p < 0.01$, $r^2 = 0.45$; Fig. 1.3a), suggesting potential recovery of denitrification since wetland restoration. Interestingly, although DEA was significantly different between restored (185 ± 43.3

ng N g⁻¹ soil hr⁻¹) and unrestored (903 ± 383 ng N g⁻¹ soil hr⁻¹) wetlands for the bottom layer; no other soil property was significantly different between restored and unrestored wetlands in the bottom layer (Table 1.1; Fig. 1.2). Microbial biomass (i.e., SIR) also increased with time since restoration (both layers $p < 0.05$, top layer $r^2 = 0.30$, bottom layer $r^2 = 0.26$; Fig. 1.3c), suggesting that the recovery of the microbial community may be related to the recovery of DEA. The significant increase in Mn with time since restoration (top layer, $p < 0.05$, $r^2 = 0.26$) may also be related to increasing trends for microbial biomass and DEA.

In contrast to DEA, C-Min was significantly higher in restored (0.1824 ± 0.0260 CO₂ C g day⁻¹ g⁻¹ soil) compared to unrestored (0.1076 ± 0.0224 CO₂ C g day⁻¹ g⁻¹ soil) wetlands (top layer, $p < 0.05$; Fig. 1.2b) and did not change significantly with time since restoration (Fig. 1.3b). Higher rates of C-Min in restored wetlands may be due to higher concentrations of NH₄⁺-N in shallow soils of restored (101 ± 14.3 mg NH₄⁺ kg⁻¹ soil) compared to unrestored (60.8 ± 11.2 mg NH₄⁺ kg⁻¹ soil) wetlands ($p < 0.05$, $t = 2.2$, $df = 29.9$; Table 1.1). There were no significant differences of microbial biomass between restored and unrestored in the top and bottom soil layer (Fig 1.2c).

Soil chemistry was similar for restored and unrestored wetlands, except for NH₄⁺-N concentrations which were significantly greater in restored wetlands (top layer, $p < 0.05$, $t = 2.17$, $df = 26.0$; Table 1.1). Restored and unrestored wetlands had similar SOM and soil EC (Table 1.1). Although metals were highly variable, they did not differ significantly between restored and unrestored wetlands. Concentration of Mn was the only soil chemistry parameter that increased with time since restoration (top soil layer, $p < 0.05$, $r^2 = 0.26$).

1.4.2 Environmental Drivers

1.4.2.1 Potential Denitrification

Best fit regression models explained 50% and 22% of variation in denitrification potential in top and bottom layers, respectively. Soil EC was negatively related to DEA, although the coefficient was not significant for the bottom layer (Table 1.2). In the top layer, microbial biomass was positively related to DEA and alone explained 35% of the variation in DEA (Tables 1.2, 1.3). Cu was also positively related to DEA for the top layer (Table 1.2). Mn was the only variable significantly positively correlated with DEA in the bottom layer (Table 1.2). Increased Mn with DEA suggests Mn may serve in a redox pair for nitrogen cycling.

1.4.2.2 Carbon Mineralization

Regression models including microbial biomass, nutrients and metals explain 74% and 58% of the variation in C-Min for top and bottom layers respectively (Tables 1.2- 1.4). Soil EC was not significantly related to C-Min. C-Min increased with microbial biomass (top layer $p < 0.001$, bottom layer $p < 0.05$) and $\text{NH}_4^+\text{-N}$ (top layer $p < 0.01$, bottom layer $p < 0.05$). Microbial biomass was a particularly important predictor of C-Min, explaining 58% and 21% of the variation in C-Min for the top and bottom layers, respectively (Table 1.2). In the top layer, C-Min decreased with increasing soil Fe content. In addition to microbial biomass and $\text{NH}_4^+\text{-N}$, SOM and Pb were also included in the best fit model for the bottom layer as significant explanatory variables (Tables 1.2, 1.4) with C-Min increasing with SOM and decreasing with Pb.

1.4.2.3 Substrate Induced Respiration

Microbial biomass significantly declined with soil EC in the bottom layer only ($p < 0.05$) and increased with SOM in the top ($p < 0.01$) and bottom layer ($p < 0.001$). Metals had a mixed effect on microbial biomass with Cu and Mn increasing microbial biomass and Pb decreasing microbial biomass (Table 1.2). Best fit regression models explained 55% and 59% of variation in microbial biomass for the top and bottom layers, respectively. SOM was a particularly important

predictor of microbial biomass, explaining 39% and 35% of variation in microbial biomass alone (Tables 1.2 - 1.4). In the top layer, microbial biomass decreased with increasing Pb ($p < 0.01$; Table 1.2) indicating potential inhibiting effects. Cu was positively related to microbial biomass only in the bottom layer ($p < 0.001$) suggesting Cu requirement for microbial growth. Similarly to DEA, Mn significantly increased microbial biomass suggesting the positive effects of Mn on DEA and microbial biomass may be related (top layer $p < 0.05$; Table 1.2). For the bottom soil layer the best fit model included significant explanatory variables SOM, soil EC and Cu.

1.5 DISCUSSION

Coastal wetlands are located at a vulnerable interface experiencing contaminated runoff and saltwater intrusion. In our field study of restored and unrestored tidal wetlands, we found both salinity and urban contamination (metals) played a role in carbon and nitrogen cycling. Salinity decreased DEA and microbial biomass, while urban metals had a mixed effect. Cu was positively correlated with DEA and microbial biomass, while Pb had a negative effect on C-Min and microbial biomass. The positive effects of Cu were only significant when soil EC was also included as a significant variable suggesting potential interaction between salinity and Cu. Predictions of sea level rise and shifts in storm and drought patterns (IPCC 2014) may increase the interaction of saltwater intrusion with urban metal contamination in the coastal landscape.

1.5.1 Site Attributes

Across all sites ($n=32$), soil Cl^- concentrations ranged from 26.8 to 51550 (mean \pm SEM, top 13432 ± 29201 mg Cl^- kg^{-1} dry soil; bottom 11433 ± 2385 mg Cl^- kg^{-1} dry soil), while SO_4^{2-} in the top layer averaged 2554 ± 463.4 mg SO_4^{2-} kg^{-1} dry soil and 2343 ± 396.6 mg SO_4^{2-} kg^{-1} dry soil in the bottom layer. NH_4^+ -N in the top and bottom layer (Table 1.1) were considerably greater than concentrations found in freshwater and saltwater coastal wetlands (Langis et al.

1991; Hopfensperger et al. 2009; McKee et al. 2016). Concentrations of Cu, Pb, and Fe (Table 1.1) were comparable to those found in a CT coastal wetland (Benoit et al. 1999) and other estuaries (Spencer and MacLeod 2002; Hu et al. 2013). Mean Cu and Pb were within the Effects Range-Low (ERL) and Effects Range-Median (ERM) suggesting metals concentrations on average are less than concentrations in which biological effects frequently occur, but within levels in which effects would occasionally occur (Long et al. 1995). Mean DEA across sites (n=32) in the top ($2222 \pm 386.7 \text{ ng N g}^{-1} \text{ soil hr}^{-1}$) and bottom layer ($521 \pm 189 \text{ ng N g}^{-1} \text{ soil hr}^{-1}$) were highly variable, but within ranges found in coastal wetlands (Findlay et al. 2003; Windham and Meyerson 2003; Dodla et al. 2008; Hopfensperger et al. 2009). C-Min in the top ($0.15 \pm 0.02 \text{ CO}_2 \text{ C g day}^{-1} \text{ g}^{-1} \text{ dry soil}$) and bottom soil layer ($0.07 \pm 0.01 \text{ CO}_2 \text{ C g day}^{-1} \text{ g}^{-1} \text{ dry soil}$) were orders of magnitude greater than rates found in inland and tidal wetlands (Ahn et al. 2009; Li et al. 2015) suggesting high carbon availability.

1.5.2 Effects of Restoration on Biogeochemistry

Our results suggest that although DEA may be lower in restored wetlands (Fig. 1.2), DEA increases with time since restoration (Fig. 1.3a), suggesting a potential recovery of DEA over time after wetland restoration. The recovery of DEA and microbial biomass over time in the top layer indicates denitrifiers may dominate the microbial biomass recovery in shallower soils (Bettez and Groffman 2012). In the bottom layer, microbial biomass increased with time since restoration, but both DEA and C-Min showed no significant recovery at depth. Microbial biomass recovery in deeper soils was likely dominated by other microbial communities (Ma et al. 2017).

Greater $\text{NH}_4^+\text{-N}$ and C-Min in restored wetlands suggest enhanced nutrient deposition or transformation resulting from restoration (Megonigal and Neubauer 2009; Das et al. 2015).

Although we found no significant difference in SOM between restored and unrestored wetlands, differences in organic material stability could play a role in regulating microbial processing rates. Our results are similar to previous studies in which restored wetland sites exhibited greater C-Min compared to reference sites (Craft et al. 2003; Glatzel et al. 2004; Lawrence et al. 2013) suggesting greater nutrient bioavailability after restoration potentially driven by shifts in plant productivity and hydrologic regime post restoration. Nitrogen mineralization, the conversion of organic nitrogen to inorganic forms (i.e. NH_4^+), is also regulated by organic material stability (Janssen 1996), suggesting bioavailability may regulate both NH_4^+ -N and C-Min in this study.

We found a significant increase in Mn with time since restoration in the top layer suggesting a potential link of Mn with microbial biomass and DEA (Fig. 1.3a,c). Mn is ubiquitous in the environment, and the concentrations found in our study are within comparable ranges found in wetland soils (Howe et al. 2004; Nath et al. 2013). Increasing Mn and microbial biomass with time with restoration indicate dissimilatory Mn reduction as a pathway of organic matter oxidation for microbial growth (Lovley and Phillips 1988; Lovley 1991; Nealson and Myers 1992; Rysgaard et al. 2001). However, less is known about coupled nitrogen cycling with Mn. The many oxidation states of Mn available for redox reactions with the nitrogen cycle offers the possibility of indirect or direct enhancement of DEA by Mn. Previous studies have described potential of Mn mediated denitrification and nitrification (Luther et al. 1997; Luther and Popp 2002; Newton 2006; Fernandes et al. 2015). Mn oxidation coupled with reduction of nitrate to N_2 (Luther et al. 1997) or reduction of Mn oxide coupled with oxidation of nitrite to nitrate (Bartlett 1981) are pathways of Mn mediated nitrogen cycling which could enhance DEA.

1.5.3 Response of Microbial Processing to Salinity

The negative effects of salinity on microbial biomass and DEA (Table 1.2) suggests broader negative effects on microbial community with some communities exhibiting more sensitivity (denitrifiers) than others. Decreased microbial biomass and DEA with salinity may be driven by many mechanisms including direct microbial and enzyme effects, formation of toxic compounds, and mobilization of nutrients. Salt can cause osmotic stress to microbes (Uygur 2006) and H₂S can have inhibitory effects on biota and certain steps of the nitrogen cycle (Joye and Hollibaugh 1995; Camargo and Alonso 2006; Pan et al. 2013b). Previous research has shown inhibition of nitrous oxide reduction to N₂ due to H₂S (Senga et al. 2006), which is supported by greater N₂O emissions with increasing SO₄²⁻ or H₂S (Brunet and Garcia-Gil 1996; Helton et al. 2014). Conversely H₂S can also serve as an electron donor for denitrifying bacteria (Schedel and Truper 1980), increasing potential for denitrification. Increased salinity enhanced the flux of NH₄⁺ to the water column which can then be exported with tides (Giblin et al. 2010; Ardón et al. 2013). Export of soil NH₄⁺ effectively decreases the N supply for coupled nitrification-denitrification in coastal wetlands. Our results show negative effect of salinity on DEA and microbial biomass, although the exact mechanisms cannot be identified.

Although the increasing concentrations of terminal electron acceptors with salinity supports findings of increasing C-Min and decreasing carbon accumulation (Chambers et al. 2011; Weston et al. 2011; Baustian et al. 2017) in our study, salinity had no effect on C-Min. Our results suggest other factors such as levels of salinity and carbon availability also play a role in regulating C-Min (Blagodatskaya and Kuzyakov 2008; Setia et al. 2011; Wang et al. 2017).

1.5.4 Response of Microbial Processing to Metals

Common urban metals, Cu or Pb, are included as significant variables in all regression models for DEA, C-Min, and microbial biomass for at least one depth interval (Table 1.2). Cu exhibited a positive effect only when soil EC also exhibited significant effects. In contrast, Pb generally reduced carbon and nitrogen cycling, but only when soil EC was not a significant variable. Our results suggest potential interaction between salinity and bioavailability of metals with a net positive effect on nitrogen processing and microbial biomass driven by Cu concentrations. Cu solubility increases with salinity (Giller et al. 1998) suggesting enhanced Cu available for denitrification metalloenzymes nirK and nosZ (Glass and Orphan 2012), although the binding of sulfides to Cu may limit Cu availability for metalloenzymes (Moffett et al. 2012). Cu is also a cofactor for many enzymes essential for cell growth (Samanovic et al. 2012). We expect increased Cu availability to enhance microbial biomass and denitrification rates unless Cu concentrations are high enough to cause toxicity effects (Holtan-Hartwig et al. 2002). Cu may also counteract negative effects of saltwater constituents on carbon and nitrogen processing. Metal-sulfide precipitation by Cu or Fe alleviates H₂S toxicity (Manconi et al. 2006; Van Der Welle et al. 2006; Bartacek et al. 2010).

Our results of negative effects of Pb on C-Min and microbial biomass have been found in previous studies (Saviozzi et al. 1997; Shang et al. 2012). Brookes and McGrath (1984) found metal pollution decreased the ratio of soil microbial carbon to soil organic carbon indicating microbial decline due to metal loading. Negative effects of lead are often associated with microbial toxicity (Khan et al. 2010; Shang et al. 2012), although in our study system of tidal wetlands the bioavailability of lead is likely minor due to pH of surface water generally greater than 7. At high pH, increased negatively charged sites (OH⁻) on soil surface and edges (Bradl

2004) result in increased capacity of metal sorption. In a controlled experiment on metal bioavailability to plants, water soluble Pb and plant uptake of Pb both decreased with increasing pH from 5 to 8 (Reddy and WHJ 1977). Pb complexation with organics may lead to less substrate availability for C-Min (Giller et al. 1998).

Fe was also found as a significant negative explanatory variable for C-Min (Table 1.2). The negative effects of Fe on C-Min may be linked to reduced substrate availability for C-Min due to sorption of organic matter to ferrihydrite (Eusterhues et al. 2008). In a study of clays and iron hydroxide, Jones and Edwards (1998) found reduced bioavailability of carbon substrates found in root exudates in the presence of clay and iron hydroxide. Coupled effects of saltwater intrusion and metal exposure on biogeochemical function of coastal wetlands is complex and further investigation is needed to identify to dominant reactions.

1.6 CONCLUSIONS

Our results suggest functional recovery after restoration for DEA, C-Min, and microbial biomass is possible. In fact, increased C-Min and NH_4^+ in restored sites suggest TFR may change availability of nutrients for carbon and nitrogen mineralization within decades. Salinity and urban metals appear to play a role in regulating carbon and nitrogen processing in coastal wetlands although further research is needed to better identify direction and magnitude of salinity effects on carbon and nitrogen processing at the landscape level. As wetland restoration and protection measures are taken, land managers need to consider how restoration in areas receiving urban runoff will respond to climate change and how these responses affect the efficacy of restoration. We recommend restoration goals be explicitly stated and prioritized prior to project implementation in order to optimize the beneficial outcomes of restoration within the desired time-scale.

Table 1.1 Mean (\pm standard error of mean) of soil chemistry parameters of restored and unrestored wetlands for the top (0-5 cm) and bottom (5-10 cm) soil layers.

Wetland	Depth	SOM	NH ₄ ⁺ -N	Soil EC	Copper	Lead	Manganese	Iron
Type	cm	%	mg kg ⁻¹	mS cm ⁻¹	mg kg ⁻¹ soil	mg kg ⁻¹ soil	mg kg ⁻¹ soil	mg kg ⁻¹ soil
Restored	0 - 5	35.0 (4.15)	101.1 ^a (14.3)	2.9 (0.70)	47.1 (8.02)	73.1 (18.3)	261.7 (82.5)	21448 (3331)
	5 - 10	32.2 (4.18)	37.8 (6.24)	2.8 (0.70)	59.9 (13.9)	96.7 (19.0)	136.9 (17.41)	17302 (2291)
Unrestored	0 - 5	32.3 (4.09)	60.8 ^b (11.2)	2.2 (0.67)	76.8 (19.9)	71.43 (14.4)	292.4(54.1)	19532 (2956)
	5 - 10	29.3 (3.89)	33.1 (6.91)	2.0 (0.62)	145.1 (51.0)	139.0 (34.1)	195.0 (26.83)	19010 (2579)

Letters denote significance ($p < 0.05$) from t test comparing restored and unrestored wetlands at the same depth interval

Table 1.2 Best fit multiple linear regression models for potential denitrification (DEA), carbon mineralization (C-Min), and microbial biomass (SIR), for the top (0-5 cm) and bottom (5-10 cm) soil layers with regression intercept, adjusted r^2 , and coefficient estimates for each parameter included in the model.

Model	DEA		C-Min		SIR	
	0-5 cm	5-10 cm	0-5 cm	5-10 cm	0-5 cm	5-10 cm
R _{adj} ²	0.50	0.22	0.74	0.58	0.55	0.59
P-value	0.00	0.03	0.00	0.00	0.00	0.00
Intercept	4.31*	-3.96	0.15	0.02	-0.21	0.13
SIR	1.66*		0.09*	0.04*		
DEA						
SOM		0.05*		0.00*	0.02*	0.01*
NH ₄ ⁺	0.58		0.04*	0.02*	0.17	-0.14
Soil EC	-0.36*	-0.31	0.01			-0.06*
Cu	1.19*					0.25*
Pb	-0.92			-0.02*	-0.21*	
Mn		2.20*			0.17*	
Fe		-0.00	-0.03*			

Table 1.3 Candidate multiple regression models for microbial process rates in the top soil layer for each possible number of model coefficients (K), including the intercept. Reported statistics include adjusted r^2 (r_{adj}^2), Mallows's C_p , Akaike's Information Criterion, the difference between the candidate and best model's AIC (Δ_i), and the residual sum of squares (RSS). Candidate models with lowest AIC are in bold and coefficients for those models are reported in Table 1.2.

Model:	K	r_{adj}^2	C_p	AIC	Δ_i	RSS
<u>Top Layer</u>						
<u>Denitrification Potential</u>						
SIR	2	0.33	8.17	30.52	6.18	73.29
SIR, Mn	3	0.39	5.60	28.19	3.85	64.02
SIR, Soil EC, Cu	4	0.43	4.42	26.80	2.46	57.58
SIR, Soil EC, Cu, Pb	5	0.48	3.19	24.94	0.60	51.04
SIR, NH_4^+, Soil EC, Cu, Pb	6	0.50	3.23	24.34	0.00	47.06
SIR, NH_4^+ , Soil EC, Cu, Pb, Mn	7	0.49	5.04	26.08	1.74	46.67
SIR, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	8	0.46	7.01	28.04	3.70	46.62
SIR, OM, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	9	0.44	9.00	30.03	5.69	46.60
<u>Carbon mineralization</u>						
SIR	2	0.56	18.53	-180.0	13.76	0.10
SIR, NH_4^+	3	0.67	7.24	-188.8	5.00	0.07
SIR, NH_4^+ , Fe	4	0.72	3.19	-193.2	0.57	0.06
SIR, NH_4^+, Soil EC, Fe	5	0.74	3.09	-193.7	0.00	0.05
SIR, NH_4^+ , Soil EC, Pb, Fe	6	0.74	3.74	-193.5	0.22	0.05
SIR, OM, NH_4^+ , Soil EC, Pb, Fe	7	0.74	5.31	-192.1	1.64	0.05
SIR, OM, NH_4^+ , Soil EC, Pb, Mn, Fe	8	0.73	7.05	-190.5	3.28	0.05
SIR, OM, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	9	0.72	9.00	-188.5	5.21	0.05
<u>Microbial Biomass</u>						
OM	2	0.35	10.63	-61.72	8.91	4.10
OM, Pb	3	0.45	5.76	-65.99	4.64	3.37
OM, Pb, Mn	4	0.50	3.79	-68.27	2.36	2.95
OM, NH_4^+, Pb, Mn	5	0.55	2.24	-70.63	0.00	2.58
OM, NH_4^+ , Pb, Mn, Fe	6	0.54	4.07	-68.85	1.78	2.56
OM, NH_4^+ , Cu, Pb, Mn, Fe	7	0.52	6.01	-66.94	3.69	2.55
OM, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	8	0.50	8.00	-64.95	5.68	2.55

Table 1.4 Candidate multiple regression models for microbial process rates in the bottom soil layer for each possible number of model coefficients (K), including the intercept. Reported statistics include adjusted r^2 (r_{adj}^2), Mallows's C_p , Akaike's Information Criterion, the difference between the candidate and best model's AIC (Δ_i), and the residual sum of squares (RSS). Candidate models with lowest AIC are in bold and coefficients for those models are reported in Table 1.2.

Model:	K	r_{adj}^2	C_p	AIC	Δ_i	RSS
Bottom Layer						
<u>Denitrification Potential</u>						
SIR	2	0.10	2.86	45.34	1.93	116.5
Mn, Fe	3	0.13	2.87	45.20	1.80	108.9
OM, Soil EC, Mn	4	0.17	2.43	44.37	0.97	99.7
OM, Soil EC, Mn, Fe	5	0.22	2.09	43.40	0.00	90.9
OM, Soil EC, Pb, Mn, Fe	6	0.21	3.50	44.62	1.22	88.7
OM, NH_4^+ , Soil EC, Pb, Mn, Fe	7	0.19	5.06	46.01	2.60	87.0
SIR, OM, NH_4^+ , Soil EC, Pb, Mn, Fe	8	0.16	7.01	47.94	4.54	86.8
SIR, OM, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	9	0.12	9.00	49.93	6.52	86.8
<u>Carbon mineralization</u>						
OM	2	0.44	8.53	-215.3	6.81	0.03
OM, Pb	3	0.48	6.87	-216.7	5.44	0.03
SIR, NH_4 , Cu	4	0.52	4.95	-218.7	3.36	0.03
SIR, OM, NH_4^+, Pb	5	0.58	2.48	-222.1	0.00	0.02
SIR, OM, NH_4^+ , Cu, Pb	6	0.59	3.30	-221.7	0.42	0.02
SIR, OM, NH_4^+ , Cu, Pb, Fe	7	0.57	5.18	-219.9	2.25	0.02
SIR, OM, NH_4^+ , Cu, Pb, Mn, Fe	8	0.56	7.00	-218.1	4.01	0.02
SIR, OM, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	9	0.54	9.00	-216.1	6.01	0.02
<u>Microbial Biomass</u>						
Cu	2	0.38	17.69	-69.06	10.61	3.26
OM, Cu	3	0.45	12.68	-72.39	7.28	2.76
OM, Soil EC, Cu	4	0.56	5.87	-78.66	1.01	2.13
OM, NH_4^+, Soil EC, Cu	5	0.59	5.19	-79.67	0.00	1.94
OM, NH_4^+ , Soil EC, Cu, Mn	6	0.59	6.06	-79.02	0.65	1.86
OM, NH_4^+ , Soil EC, Cu, Mn, Fe	7	0.61	6.01	-79.64	0.03	1.72
OM, NH_4^+ , Soil EC, Cu, Pb, Mn, Fe	8	0.59	8.00	-77.66	2.01	1.71

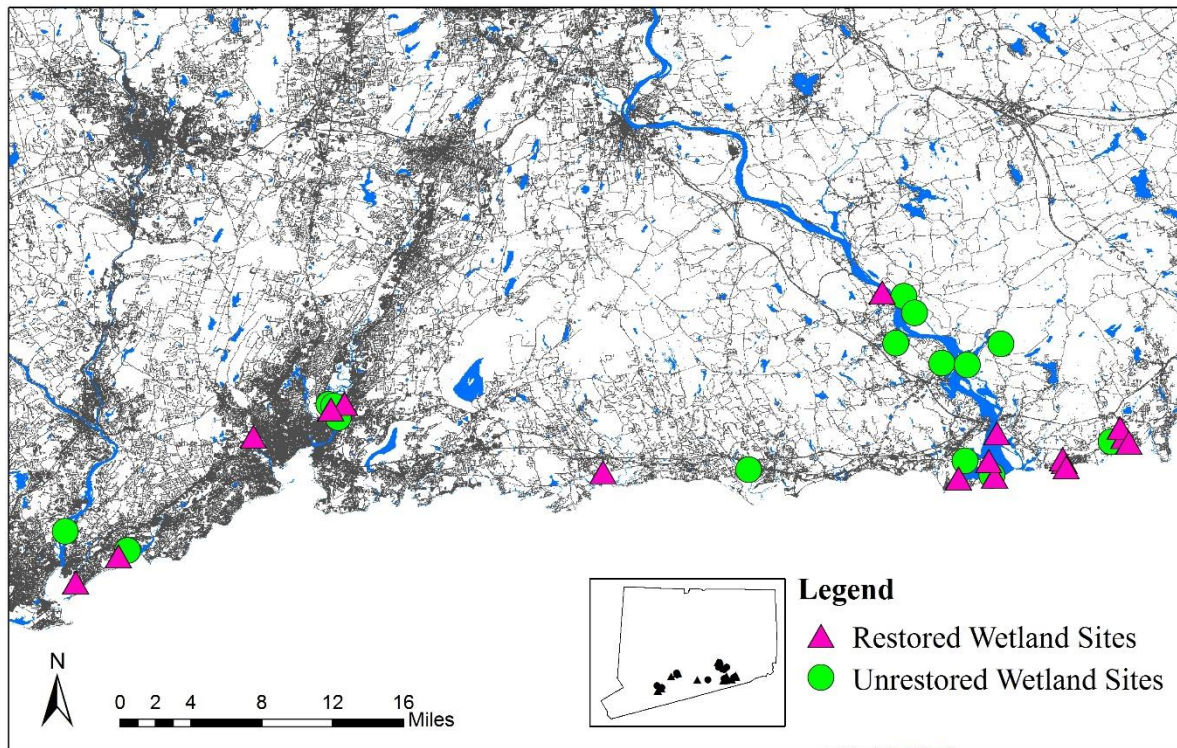


Figure 1.1 Wetland sampling site locations in Connecticut with developed land cover (grey) and water (blue) from 2010 land cover data (CLEAR 2010).

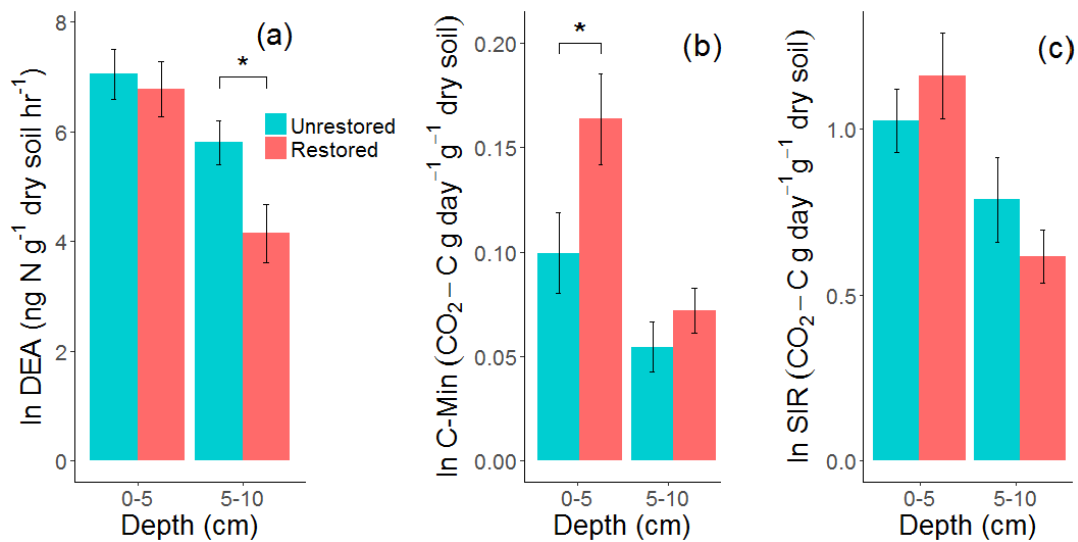


Figure 1.2 Comparison of unrestored and restored wetlands for potential denitrification rates (a) carbon mineralization rates (b) and microbial biomass (c) at corresponding depth intervals. * $p < 0.05$

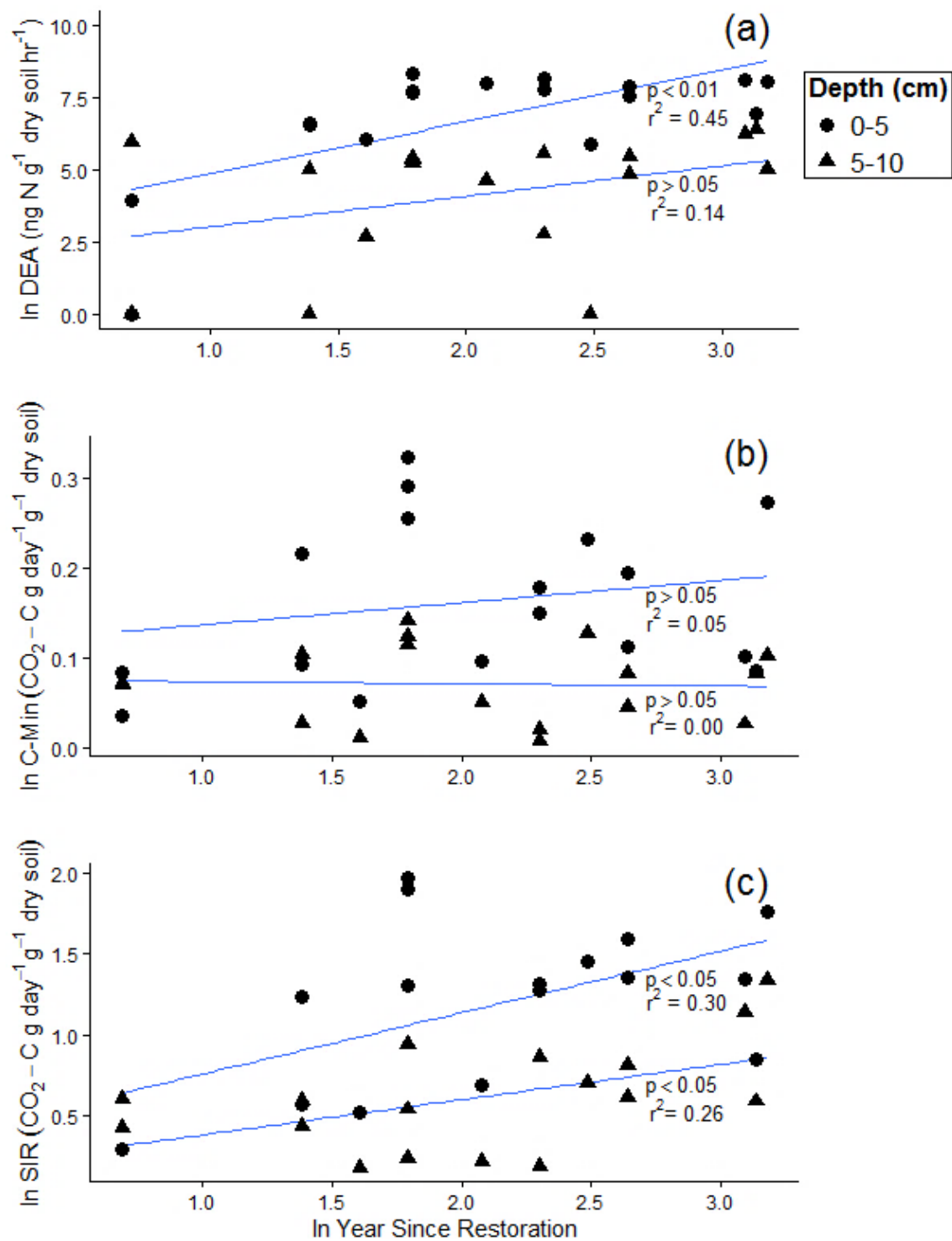


Figure 1.3 Simple linear regression for potential denitrification rates (a) carbon mineralization rates (b) and microbial biomass (c) with year since restoration.

Chapter 2. When urban runoff meets saltwater intrusion: carbon and nitrogen cycling in a wetland soil core experiment

2.1 ABSTRACT

Wetlands serve an important role in regulating greenhouse gases (GHGs) globally.

Anthropogenic stressors including contaminant loading from runoff and saltwater intrusion have the potential to alter GHG emissions, particularly in tidal wetlands. In July 2016, we collected 45 intact soil cores from a tidal wetland in Lyme, Connecticut, U.S.A. for a mesocosm experiment.

Our goal was to disentangle the effects of saltwater intrusion (elevated salinity versus elevated sulfate) and runoff (elevated nitrogen versus elevated copper) on carbon and nitrogen cycling.

We applied treatments to examine the effects of urban runoff (i.e., elevated nitrate, elevated copper, elevated nitrate and copper), saltwater intrusion (i.e., elevated sulfate, elevated salinity, and elevated sulfate and salinity), and the combined effects of urban runoff and saltwater intrusion (elevated nitrate, copper, salinity, and sulfate). We measured GHG fluxes (CO_2 , CH_4 , N_2O) eight times and soil pore water chemistry nine times over seven weeks, including pre-treatment. Soils were also harvested at the end of the experiment to analyze soil chemistry and microbial carbon and nitrogen processing. We found that copper, saltwater, and the combined effects of urban runoff and saltwater intrusion significantly decreased CO_2 flux compared to the freshwater control ($p < 0.05$) suggesting salt and copper toxicity reduces CO_2 flux from wetland soils. Carbon mineralization (C-Min) in the top 5 cm in soil harvested at the end of the experiment showed similar patterns with treatment. Compared to freshwater control CH_4 fluxes, fluxes were lower for the copper treatment for the most dates ($n=3$), followed by runoff ($n=1$) and sulfate ($n=1$). Compared to pre-treatment flux, N_2O was significantly higher for the saltwater, seawater, and combined urban runoff and saltwater intrusion treatments for five, two, and one post-treatment dates, respectively ($p < 0.05$). Increasing N_2O flux followed similar

patterns of increasing pore water NH_4^+ over time, suggesting salt induced NH_4^+ mobilization as the mechanism driving increasing N_2O . Our study indicates urban runoff and saltwater intrusion synergistically reduce CO_2 flux while also increasing N_2O over time.

2.2 INTRODUCTION

Coastal wetlands are susceptible to effects of land development and climate change (Carpenter et al. 1998; Small and Nicholls 2003; Burns et al. 2005; IPCC 2014). Coastal population density has steadily increased in recent decades and is projected to continue to rise across the globe (Crossland et al. 2005; Eurostat 2010; Wilson and Fischetti 2010). Associated land development directly reduces wetland habitat and degrades wetlands due to elevated nutrients and metals in urban and agricultural runoff (Carpenter et al. 1998; Bergback et al. 2001; 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 (Nicholls and Cazenave 2010; Werner et al. 2013; Herbert et al. 2015). Because wetlands are located in a transitional area between land and water, they receive runoff from upstream developed land and saltwater intrusion from downstream coastal systems, creating biogeochemical regime shifts (Helton et al. 2014) that may drive changes in wetland ecosystem function.

Wetlands cover less than 10% of the Earth's surface (Mitsch and Gosselink 2015), but are estimated to store 20-30% of the Earth's soil pool of carbon (Mitsch et al. 2013). Globally, wetlands are considered net carbon sinks (Bridgman et al. 2006; Mitsch et al. 2013), but wetlands are also sources of CH_4 , with global warming potential (GWP) 28 times of CO_2 over 100 years (Whalen 2005; IPCC 2014). Through denitrification, wetlands provide nitrogen

removal from the aquatic ecosystem, although N_2O , a greenhouse gas with GWP 265 times of CO_2 , can be produced in the process (Ward 2013; IPCC 2014; Mitsch and Gosselink 2015). In a review of nitrogen retention in wetlands, lakes, and rivers, in North America and Europe, Saunders and Kalff (2001) found wetlands removed nearly twice the amount of nitrogen compared to lakes, primarily via denitrification. As saltwater intrusion increases along developed coastal areas, understanding the response of wetlands to changing chemical regimes will be important for predicting future carbon retention and greenhouse gas production in coastal wetlands.

Elevated nitrogen in runoff from developed land (Taylor et al. 2005; Hwang et al. 2009) can affect carbon and nitrogen processing in wetlands. Increased nitrogen loading is commonly associated with greater rates of denitrification (Wallenstein et al. 2016) and increased N_2O emissions (Hefting et al. 2003; Liu and Greaver 2009; Moseman-Valtierra et al. 2011) indicating wetlands play an essential role in nitrogen removal, but also emit a potent greenhouse gas in the process. Nitrogen additions may have variable effects on carbon gas emissions. Nitrogen additions did not significantly affect CO_2 or CH_4 production in laboratory incubations or in-situ fertilization experiments (Keller et al. 2005; Min et al. 2011; Moseman-Valtierra et al. 2011), but increased CH_4 emissions in rice fields and terrestrial systems (Liu and Greaver 2009; Cheng-Fang et al. 2012) potentially due to reduced soil CH_4 uptake with increasing nitrogen additions (Liu and Greaver 2009).

Industrial effluent and urban runoff contributed to metal enrichment, of copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) above natural levels (Yang et al. 2013; Bebianno et al. 2015; Martínez-Santos et al. 2015). In Chapter 1, we found Cu was positively correlated with denitrification potential and microbial biomass in coastal wetland soil cores. Many microbial

processes, including denitrification and methane oxidation (Tavares et al. 2006; Samanovic et al. 2012; Vita et al. 2016), require metals in enzymes (Giller et al. 1998; Glass and Orphan 2012). This suggests the potential for elevated Cu may to increase specific microbial processing rates. Despite metal requirement for microbial metalloenzymes, metals are generally considered toxic at higher levels (Özbelge et al. 2007) and are associated with chronic toxicity in estuarine sediments (Ochoa-Herrera et al. 2009; Cruz et al. 2014). Threshold toxicity of Cu to biological systems can range in orders of magnitude (Özbelge et al. 2007). Depending on the type of sediment, treatments of Cu had insignificant or inhibitory effects on denitrification (Magalhães et al. 2007). Other studies indicated Cu suppressed denitrification (Holtan-Hartwig et al. 2002; Magalhães et al. 2011), although microbes can develop metal tolerance over time (Giller et al. 1998). Additionally, lower rates of carbon mineralization (C-Min) were associated with increased Cu concentrations (Nwuche and Ugoji 2008; Luke et al. 2015),.

Saltwater intrusion due to sea level rise or other anthropogenic factors increases the salinity of wetlands (Herbert et al. 2015). Research on the effects of elevated salinity on carbon and nitrogen cycling in wetlands has produced contrasting results. Across a natural gradient, Marton et al. (2012) found increased salinity associated with increased CO₂ production. These results are consistent with increased CO₂ production after seawater additions in short term laboratory experiments (Chambers et al. 2011; Neubauer et al. 2013). In contrast, long term (3.5 years) in-situ seawater additions treatments reduced soil CO₂ production and increased C:N ratios suggesting shifts in C availability affects CO₂ production (Neubauer et al. 2013). In the same study, seawater treatments for short term incubations and long term in-situ studies reduced CH₄ production. Decreased CH₄ production due to competition between methanogens and sulfate reducing bacteria for available organic carbon is generally recognized as the main pathway of

reduced CH₄ flux with increasing salinity (Bartlett et al. 1987b; Marton et al. 2012; Herbert et al. 2015), although additions of NaCl also reduced CH₄ production over a two week incubation (Chambers et al. 2011) potentially due to ionic stress.

There is still uncertainty of the predominant mechanisms driving changes in nitrogen cycling from increasing salinity. Across a natural gradient, increasing salinity decreased denitrification (Rysgaard et al. 1999; Seo et al. 2008; Craft et al. 2009) or had no effect (Marton et al. 2012). Salt treatments had no effect (Magalhaes et al. 2005), or increased and decreased denitrification depending on salinity levels (Marks et al. 2016). In coastal plain wetlands, N₂O production was positively correlated with sulfate (SO₄²⁻) concentrations (Helton et al. 2014). Similar results were found as higher metal sulfide concentrations enhanced production of N₂O due to sulfide inhibition of nitrous oxide reductase (Brunet and Garcia-Gil 1996).

The potential complexity and overwhelming inconsistency of the effects of runoff and saltwater intrusion on carbon and nitrogen cycling highlight a need for a basic understanding of the mechanisms driving these changes. Some studies have begun to parse the effects of elevated salinity and sulfate (Seo et al. 2008; Chambers et al. 2011; Marton et al. 2012) or the effects of runoff and saltwater intrusion on carbon and nitrogen cycling of wetlands (Helton et al. 2014; Hu et al. 2016), but more research is necessary in order to predict how wetland carbon and nitrogen cycling will respond to multiple anthropogenic stressors. Therefore, the objective of this study was to disentangle the effects of runoff (elevated nitrate and copper) and saltwater intrusion (elevated salinity and sulfate) on carbon and nitrogen cycling in coastal wetlands. To address this objective we conducted an experiment in which intact soil cores received eight treatments, intended to identify the effects of chemical constituents of seawater (elevated salinity, elevated sulfate, and elevated salinity and sulfate), runoff (elevated nitrate, elevated copper, and elevated

nitrate and copper), and the combined effects of runoff and seawater intrusion (Table 2.1). We measured carbon (CO₂, CH₄) and nitrogen (N₂O) gas fluxes over the seven week experiment and potential denitrification, carbon mineralization, and microbial biomass at the end of the experiment.

2.3 METHODS

2.3.1 Study Site and Field Sampling

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) (Fig. 2.1). This wetland is at the forefront of saltwater intrusion as it is within the saltwater limit along the CT River (CT DEEP 1995).

Soil samples were collected at low tide along the unvegetated bank of the channel. We recorded surface water salinity and temperature with a handheld meter (model 556 MPS, YSI Inc., Yellow Springs, OH) and site conditions with a handheld weather station (Kestrel Meters, Minneapolis, MN). At time of sampling, surface water salinity was 4.09 ppt, although during previous visits surface water salinity ranged from 0.22 ppt 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 period (NOAA National Centers for Environmental Information 2016).

2.3.2 Experimental Design

Soil cores were taken to the laboratory for an experiment in which eight treatments were applied for seven weeks (Table 2.1). We designed the experiment to identify the individual and combined effects of chemical changes due to saltwater intrusion and runoff on solute mobilization and carbon and nitrogen processing. Levels of salinity, nitrate (NO₃⁻), and Cu were

chosen based on environmentally relevant concentrations (Table 2.1). Artificial seawater (ASW) and artificial freshwater recipes (AFW) were used for treatments and as matrices for combination treatments (Table 2.1). The seawater treatment (18 ppt) represented the upper threshold of the mesohaline salinity class (Cowardin et al. 1979). The salt treatment (18 ppt) was made without SO_4^{2-} to identify effects of salinity alone. To identify effects of SO_4^{2-} without excess salinity, we prepared the sulfate treatment with sulfate concentrations equivalent to those found in the seawater treatment. The nitrate treatment was prepared to 2 mg N L^{-1} to represent nitrate river export in addition to storm water concentrations (Pitt 2015; Mullaney 2016). The copper treatment was prepared to 100 ug Cu L^{-1} to represent levels found in storm water (Odnevall Wallinder et al. 2009; Pitt 2015). All treatments included 4 mg C L^{-1} of carbon as potassium acetate to provide carbon that would normally be supplied by river export (Details of treatments are included in Table 2.1).

After collection, the 45 cores were immediately sealed with a bottom cap. For forty soil columns, 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 pore water extraction. During installation, we applied equal amounts of AFW as needed to keep soil cores inundated. After two weeks of equilibration, we harvested five soil cores (ambient) to analyze initial soil characteristics. For the remaining 40 cores, over the seven treatment weeks, equal volumes (30 mL) of each treatment were added to the cores three times a week (Table 2.1). Each treatment was applied to five replicate cores (5 cores per treatment, 8 treatment, 40 total cores). Soil cores were kept inundated with about 2.5 cm of overlying water. To balance water level, we drained 30 mL from each core two times a week. Every other week, we skipped one day of drainage to mitigate for evaporation loss.

We collected soil pore water prior to treatment (Week 0), 3 days (Day 3) after initial treatment, and weekly (Weeks 1-7) for the following seven weeks for a total of nine soil pore water sampling periods. We measured greenhouse gas fluxes prior to treatment (from three cores of each treatment) and weekly after initial treatment for the following seven weeks (for all five cores in each treatment) for a total of eight greenhouse gas sampling periods (Weeks 0-7).

At the end of the experiment we harvested soil cores. For each core, surface soils were sectioned into 0-5 cm and 5-10 cm depths, sieved (2 mm), and homogenized. Soil samples were analyzed at both depth intervals for soil chemical properties and carbon and nitrogen processing rates.

2.3.3 Greenhouse Gas Flux Rates

During sampling, we created an air tight chamber by enclosing the columns with a PVC end cap greased with petroleum jelly and fitted with a gas sampling port. Headspace fluxes were collected from each soil column over three 20-minute intervals by injecting 22 mL of CO₂-free air into the headspace, mixing the headspace gas by pumping the syringe three times, 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 (GC) equipped with an electron capture detector (ECD) and flame ionization detector (FID).

Gas concentrations were corrected for CO₂-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). We calculated the minimum detectable concentration difference (MDCD) for CO₂ and CH₄ with sample pairs of standards and for N₂O with pairs of ambient air samples (Yates et al. 2006). If

the change in concentration over the full incubation was less than MD CD, we set the flux to zero. For changes in concentration above MD CD, we used the slope of the regression between gas concentration and time over the forty minute incubation (Holland et al. 1999). We excluded fluxes from further analysis with poor linear relationships (i.e., $r^2 < 0.75$). We excluded 6 CO₂, 57 CH₄, and 69 N₂O fluxes (out of 304). Gas fluxes were highly variable before treatments were applied. Therefore, gas fluxes were normalized by subtracting the pre-treatment gas flux from each gas flux collected after treatments were applied (Δ gas flux).

2.3.4 Soil and Pore Water Chemistry

Ambient soil cores (n = 5) and soil cores harvested at the end of the experiment (n = 40) were dried at 105°C to determine moisture content and combusted at 550°C to determine soil organic matter (SOM) by the loss on ignition method (adapted from USDA-NRCS 1996). We extracted soil nutrients, ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N), with 2M KCl (soil:KCl = 1:10) (adapted from Keeney and Nelson 1982). KCl extractable and pore water NH₄⁺-N was determined by the phenate method (APHA 1999) and KCl extractable NO₃⁻-N by colorimetric determination of NO₃⁻-N plus nitrite (NO₂⁻-N) by enzymatic reduction (Campbell et al. 1997; Patton and Kryskalla 2011). KCl extractable and pore water NH₄⁺-N and KCl extractable NO₃⁻-N were analyzed on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT). Pore water NO₃⁻ was analyzed on a Dionex Ion Chromatography System (ICS)-1100 (Thermo Fisher Scientific, Waltham, MA). For Week 0, 80% of pore water NO₃⁻ was below detection limit (0.021 mg L⁻¹). For the following sampling weeks, all of the samples were below detection limit (detection limits ranged from 0.032 to 0.17 mg L⁻¹). All samples were also below detection for soil KCl extractable NO₃⁻-N (0.83 mg L⁻¹). Therefore pore water NO₃⁻ and

soil NO_3^- -N were excluded from statistical analysis. All pore water and soil NH_4^+ -N were above detection limit (detection limits ranged from 0.045 to 0.12 mg L⁻¹).

Water extractable (soil: water = 1:10) and pore water salt anion concentrations (i.e. chloride (Cl^-) and sulfate (SO_4^{2-})) were analyzed on the ICS. All pore water and soil Cl^- were above detection limit (0.07 – 1.0 mg L⁻¹). 99% of pore water and soil extractable SO_4^{2-} were above detection limit (0.03 – 0.87 mg L⁻¹).

Total soil Cu was determined by acid digestions with 70% HNO_3 (trace metal grade) and 30% H_2O_2 according to Method 3050B (US EPA 1996). Soil and pore water metal concentrations were analyzed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7700x with He collision cell, Agilent, Delaware, USA).

2.3.5 Carbon and Nitrogen Processing Rates

We used the acetylene block technique to measure denitrification enzyme activity (DEA) (Groffman et al. 1999). We weighed five grams of soil into a 125 mL Erlenmeyer flasks and amended soils with carbon (glucose), nitrate (KNO_3), and chloramphenicol to indicate potential denitrification rates. Flasks were sealed with Suba-Seal® septa and made anoxic with N_2 prior to gas collection. Headspace N_2O was collected from the incubations at an initial time point (acetylene injected) and for 30 minute increments thereafter (N_2 injected) for three time points total. Samples were injected into pre-evacuated vials and analyzed on the GC for N_2O . We calculated N_2O MDCD with pairs of gas standards (Yates et al. 2006). We used MDCD and $r^2 > 0.85$ to determine fluxes to keep or discard according to details specified in the ‘Greenhouse Gas Flux Rates’ section. DEA was calculated as the linear rate of evolved N_2O -N over time per gram of dry soil.

We measured CO₂ accumulation of field moist soils over a three day incubation to determine rates of aerobic C-Min. For each sample, five grams of soil were weighed into a 100 mL serum bottle and sealed with a septum and aluminum cap. Headspace samples were collected at an initial time point, and after one and three days. Headspace CO₂ was sampled by injecting five mL of CO₂-free air into the serum bottle, mixing the headspace, and injecting the five mL sample into a LI-840A CO₂/H₂O Gas Analyzer (LI-COR, Lincoln, NE) for CO₂ concentration.

We used the substrate induced respiration (SIR) method (Anderson and Domsch 1978; West and Sparling 1986) as a surrogate for soil microbial biomass. SIR is positively correlated with total microbial biomass (Britam and Neely 1990) and is commonly used as an indirect measurement of total microbial biomass (Bååth and Anderson 2003). Five grams of soil and 10 mL of yeast solution, delivering 20 mg yeast per gram of soil, were added to a 40 mL amber vial and sealed with a septum and cap. Headspace CO₂ was sampled similarly to C-Min but with a one mL injection over an initial time point, after two, and four hours. C-Min and SIR rates were calculated as the linear rate of accumulated CO₂-C over time per gram of dry soil.

2.3.6 Statistical Analysis

Statistical analyses were performed using R version 3.3.2 (R Core Team 2016), with R Studio Interface version 1.0.136 (R Studio Team 2016). Normality was tested with the Shapiro-Wilk Normality Test (R function 'shapiro.test()'). All parameters were square root or natural log (ln) transformed to improve normality. We used one-way analysis of variance (ANOVA) (R function 'aov()') to compare soil and pore water nutrients, salts, and metals, and microbial processing rates among treatments at each soil depth. To compare GHG fluxes and pore water chemistry between treatments over time, we conducted repeated measures analysis with a linear mixed effects model from the nlme package (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.

2.4 RESULTS

2.4.1 Pore Water and Soil Chemistry

As expected, pore water Cl^- concentrations for treatments with elevated salinity (saltwater, seawater, and combined) and SO_4^{2-} concentrations for treatments with elevated sulfate (sulfate, seawater, and combined) increased with time compared to treatments without added salt or sulfate, respectively (Fig. 2.3a, b; Table 2.4). Post hoc analysis indicated pore water Cl^- and SO_4^{2-} were significantly greater for treatments with elevated salinity and sulfate, respectively, compared to the freshwater control for the last six sampling weeks ($p < 0.05$), but not the first three. This is because the rate of mixing and diffusion down the soil core likely regulate amount of time for treatments to alter pore water chemistry.

Similar to pore water chemistry, soil extractable Cl^- at the end of the experiment was greater for treatments with elevated salinity compared to the freshwater control for the top and bottom soil layers (ANOVA, top $F=166.6$, $p < 0.05$; bottom $F=102.3$, $p < 0.05$; Table 2.2). In the top layer, soil extractable SO_4^{2-} was significantly greater for the sulfate treatment compared to all other treatments (ANOVA, $F=6.23$, $p < 0.05$) suggesting other constituents (salts) of combined and seawater treatments reduced mobility of SO_4^{2-} . In the bottom layer, soil extractable SO_4^{2-} was significantly greater in the sulfate treatment compared to nitrate, copper, and saltwater treatments (ANOVA, $F=4.55$, $p < 0.05$). The sulfate treatment did not have significantly different soil extractable SO_4^{2-} than the freshwater treatment although ($p = 0.09$; Table 2.2).

Pore water NO_3^- and soil extractable NO_3^- -N were typically below detection limit (see Methods Section Soil and pore water chemistry) for all treatments, including treatments with added nitrate suggesting added NO_3^- was sorbed to soil or reduced to other forms of nitrogen. Cu was typically below detection and not significantly different across treatments throughout the experiment (Fig. 2.3d; Table 2.4) suggesting Cu accumulation in the soil.

The elevated salinity of saltwater, seawater, and combined treatments mobilized higher concentrations of NH_4^+ -N into pore water likely due to cationic exchange with sea salts. For the first three sampling events (Week 0, Day 3, Week 1) there was no significant differences of pore water NH_4^+ -N between treatments suggesting effects of treatments on pore water NH_4^+ -N do not occur immediately. Pore water NH_4^+ -N concentrations generally peaked for saltwater, seawater, and combined treatments between Week 2 and Week 4 and then declined (Fig. 2.3c). On Week 2, the saltwater, seawater, and combined treatments had significantly greater pore water NH_4^+ -N compared to freshwater ($p < 0.05$). The saltwater treatment had the greatest pore water NH_4^+ -N (Fig. 2.3c). By the last pore water sampling period (Week 7) the freshwater control did not significantly differ in pore water NH_4^+ -N concentrations from saltwater, seawater, or combined treatment suggesting only temporary effects of increased salinity on mobilization of NH_4^+ -N. By Week 7, the runoff treatment had significantly lower NH_4^+ -N compared to all the treatments except for nitrate and copper (Fig. 2.3c). Although the nitrate and runoff treatments contained nitrate, these treatments did not exhibit significantly greater pore water NH_4^+ -N compared to the freshwater control over the course of the experiment. At the end of the experiment, there was a significant main effect of treatment on soil extractable NH_4^+ -N in the top and bottom layers (top layer $df=7$, $F= 5.0$, $p < 0.05$; bottom layer $df=7$, $F= 4.73$, $p < 0.05$; Table 2.2), although post-hoc analysis indicated no significant difference of freshwater treatment NH_4^+ -N compared to the

other treatments. In contrast, the nitrate treatment had the highest soil extractable $\text{NH}_4^+\text{-N}$ in the top and bottom layer (Table 2.2), suggesting $\text{NH}_4^+\text{-N}$ accumulated in soils rather than being mobilized.

SOM was significantly different across treatments for the top and bottom layers (ANOVA, top layer $F= 5.92$, $p< 0.05$, bottom layer $F= 2.74$, $p< 0.05$; Table 2.2). In the top layer, SOM was highest for sulfate and saltwater treatments and lowest for nitrate, copper, and runoff for sulfate and saltwater. The freshwater treatment was not significantly different than any treatment. The sulfate treatment also had the highest SOM in the bottom layer (Table 2.2) in which the sulfate treatment had significantly greater SOM compared to freshwater, copper, and runoff treatments (ANOVA, $p< 0.05$; Table 2.2).

2.4.2 Carbon Gas Flux and Mineralization

Saltwater, copper, and combined treatments generally exhibited a decreasing trend in CO_2 flux over the experiment (Fig. 2.2a). CO_2 flux for the saltwater and combined treatments were significantly lower than freshwater flux for 7 weeks, followed by copper ($n=6$), sulfate ($n=3$), and seawater ($n=1$) ($p< 0.05$; Fig. 2.2a). At week 1, the saltwater and copper treatments had significantly reduced CO_2 flux compared to the freshwater control treatment ($p< 0.05$; Fig. 2.2a; Table 2.3). By week 7, the final sampling week, CO_2 flux for saltwater, copper, and combined treatments were reduced compared to the freshwater control ($p< 0.05$). Over the seven weeks, the CO_2 flux for freshwater, seawater, and nitrate did not significantly change between dates.

Treatment effects were significantly different between freshwater and other treatments starting at Week 4, in which CH_4 flux for copper was significantly lower than freshwater. The copper treatment had significantly lower CH_4 flux for the most dates ($n=3$), followed by runoff

(n=1), and sulfate (n=1) ($p < 0.05$; Fig. 2.2b). Generally, the freshwater and nitrate treatments had greater CH_4 flux over the experiment, while copper and runoff treatments had lower CH_4 fluxes (Fig. 2.2b; Table 2.3), suggesting Cu and not nitrate caused lower CH_4 in the group of runoff treatments. CH_4 flux for the nitrate treatment was significantly greater than for copper for three dates ($p < 0.05$), although CH_4 flux for nitrate was not significantly different than freshwater on these dates. There was a spike in CH_4 flux during Week 2 for the nitrate treatment. For this week, CH_4 flux for nitrate was significantly greater than sulfate, seawater, nitrate and runoff ($p < 0.05$). We do not suspect this is a result of ebullition since spike in CH_4 flux are also apparent for the copper and combined treatments (Fig. 2.2b).

Aerobic C-Min was lower for saltwater, seawater, copper, runoff, and combined compared to the freshwater treatment (ANOVA, top layer, $p < 0.05$; Fig. 2.4a). Although this result is consistent with reduced CO_2 flux for saltwater, copper, and combined treatment during the last sampling week, lower C-Min for seawater and runoff treatments is not supported by CO_2 flux over the experiment (Fig. 2.2a). This discrepancy may be due to the type of C-Min incubation. The C-Min incubation performed was an aerobic incubation, but CO_2 flux may be emitted from aerobic and anaerobic metabolism. We also found reduced microbial biomass for the runoff and combined treatments compared to the freshwater control (top layer; ANOVA, Fig. 2.4c, d). C-Min rates were lower in the bottom layer compared to the top layer with fewer statistical differences among treatments. In the bottom layer, C-Min was lower for sulfate and combined treatments compared to freshwater (ANOVA, $p < 0.05$; Fig. 2.4a, b). In the bottom layer, microbial biomass was also reduced for sulfate, seawater, copper, runoff, and combined treatments (Fig. 2.4d).

2.4.3 N_2O Flux and Denitrification

N_2O was significantly higher than pre-treatment flux in the saltwater treatment for the most dates ($n=5$), followed by seawater ($n=2$), and the combined treatment ($n=1$) ($p < 0.05$); the freshwater control N_2O flux did not significantly change over time. N_2O flux of saltwater and seawater treatments were highly variable, but generally increased over the experiment compared to the freshwater control (Fig. 2.2c; Table 2.3). There were significant main effects of treatment and date for N_2O flux, but the interaction was not significant (Table 2.3). Although the saltwater, seawater, and combined treatments had the highest N_2O fluxes, they were not significantly different than the freshwater treatment for each week. On Week 4, the seawater and combined treatments had greater N_2O flux compared to the runoff treatment. Unexpectedly, nitrate containing treatments (nitrate, runoff, and combined) did not exhibit significantly greater N_2O flux compared to the freshwater or copper. DEA in soils harvested at the end of the experiment were not significantly different between treatments for the top or bottom layers (Fig. 2.4e, d).

2.5 DISCUSSION

2.5.1 *Effects of Saltwater Intrusion*

2.5.1.1 Carbon Cycling

Our results suggest that elevated salinity lowered CO_2 flux over the duration of the experiment. Our results of decreased CO_2 flux are supported by lower C-Min in the top layer for the saltwater treatment ($p < 0.05$; Fig. 2.4a), although results are not consistent with microbial biomass, which was not significantly different for saltwater compared to freshwater (Fig. 2.4c, d). Previous research reports contrasting results on the effects of salts (without sulfate) on CO_2 production. NaCl additions reduced CO_2 in clayey soils (Nourbakhsh and Sheikh-Hosseini 2006), while NaCl had no effect on CO_2 in peat soils (Chambers et al. 2011). The salt induced decrease in CO_2 flux may be due to microbial osmotic stress (Yan et al. 2015) or effects on

enzymes. Higher salinity was correlated with reduced beta-glucosidase enzyme activity (Tripathi et al. 2007; Pan et al. 2013a). The beta-glucosidase enzyme is a predominant soil enzyme used as a soil indicator of carbon cycling and soil health (Das and Varma 2011; Zhang et al. 2015). This enzyme aids in the degradation of cellulose to glucose (Singhania et al. 2013); therefore, decreased activity can result in reduced carbon availability for mineralization. If salinity were the primary driver of decreased CO₂ flux, we would expect lower CO₂ flux for the seawater treatment. Compared to freshwater CO₂ flux, flux for seawater treatment was lower for only one sampling week suggesting combined effects of elevated salt and elevated sulfate do not decrease CO₂ as consistently compared to elevated salinity or elevated sulfate alone.

Previous studies show decreased CH₄ with increasing salinity (Bartlett et al. 1987b; Chambers et al. 2011; Marton et al. 2012; Neubauer et al. 2013). In contrast, in our experiment, elevated sulfate did not typically decrease CH₄ flux compared to freshwater (Fig. 2.3b). Pore water concentrations of SO₄²⁻ steadily increase in the sulfate, seawater, and combined treatment (Fig. 2.3b), but there is no significant effect on CH₄ flux except for the sulfate treatment for the final sampling week. With higher SO₄²⁻ concentrations, sulfate reduction is more energetically favorable than methanogenesis (Megonigal et al. 2004), although our results are not consistent with this hierarchy of metabolic pathways. This suggests other physiochemical parameters, including substrate affinity (Megonigal et al. 2004), regulate the out competition of sulfate reduction compared to methanogenesis in high sulfate environments.

2.5.1.2 Nitrogen Cycling

Compared to pre-treatment flux, N₂O flux was significantly higher for the most dates in the saltwater treatment. This may be a result of preferential salt stress to the nitrous oxide reductase enzyme. Menyailo et al. (1998) found higher salt sensitivity of the nitrous oxide

reductase compared to other enzymes necessary for denitrification. Also, increasing concentrations of NaCl additions increased N₂O emissions.

Although N₂O fluxes were not significantly different between treatments over time, they follow patterns of pore water NH₄⁺ (Fig. 2.2c, Fig. 2.3c) with peak N₂O flux and pore water NH₄⁺ between Week 2 and Week 4 for the saltwater, seawater, and combined treatments. Pore water NH₄⁺ was positively related to N₂O flux over the experiment ($p < 0.05$). Similar patterns suggest coupling of increased pore water NH₄⁺ with N₂O flux. Higher NH₄⁺ mobilization for saltwater, seawater, and combined treatments is likely due to displacement of NH₄⁺ with salt cations (Rysgaard et al. 1999; Ardón et al. 2013). Salt induced displacement of NH₄⁺ to pore water potentially increases nitrogen available for coupled nitrification-denitrification, consequently increasing N₂O emissions with saltwater intrusion.

Treatments did not have a significant effect on DEA in the top and bottom layer (Fig. 2.4e, f). These results agree with previous studies in which salt treatments did not significantly affect N₂O production or denitrification (Magalhaes et al. 2005; Marton et al. 2012) although other studies indicate increased N₂O with increasing salinity attributed to inhibition of the last step of denitrification (Senga et al. 2006; Kong 2015).

2.5.2 Runoff Effects

2.5.2.1 Carbon Cycling

Copper reduced CO₂ flux for the majority of the sampling weeks suggesting suppression of C-Min or stimulation of CO₂ consumption. Decreased CO₂ flux with the copper treatment is supported by lower C-Min (Fig. 2.4a) at the end of the experiment. CO₂ flux and C-Min suppression via Cu toxicity is consistent with prior research. Cu had the highest inhibitory effect

on soil respiration compared to other heavy metals (i.e. Ni, Cd, Mn, Pb, Zn) (Saviozzi et al. 1997) and DTPA (plant available) extractable and water soluble Cu were negatively correlated with microbial biomass C, basal respiration, SIR and fluorescein diacetate activity (Bhattacharyya et al. 2008). The magnitude of C-Min response to different levels of Cu dosing is not consistent across studies (Giller et al. 1998; Rajapaksha et al. 2004). Contradictory results may arise due to toxicity decreasing the population and consequently decreasing respiration, while the death of microbes might supply more substrate for respiration and thus increase respiration (Giller et al. 1998). Cu toxicity likely drives the decreases CO₂ flux and C-Min in our study rather than stimulation of CO₂ consumption. Cu stimulation of methanogenesis is unlikely as metalloenzymes requiring Cu have not been identified for the methanogenesis pathway (Glass and Orphan 2012).

Similarly to CO₂ flux, CH₄ flux was significantly decreased in the copper treatment (Fig. 2.3b), but only for the final three sampling weeks suggesting delayed effects of copper. Lower CH₄ flux can be explained by two pathways: decreased CH₄ production or enhanced CH₄ consumption. Sanchez et al. (1996) found high concentration of Cu decreased methane production and the number of methanogens with 50% inhibition for Cu levels <10 - 250 mg/L. These levels are much higher than applied in our study (Table 2.1). Additionally, high levels of Cu decreased acetoclastic and hydrogenotrophic methanogenesis (Karri et al. 2006). Lower CH₄ can also be explained by increased methanotrophy. Methanotrophic enzymes utilize Cu (Glass and Orphan 2012) suggesting CH₄ fluxes may be decreased due to increased methanotrophy. In environments of abundant Cu, methanotrophic metalloenzymes utilize Cu to catalyze the first step of CH₄ oxidation. Methanotrophs oxidize CH₄ as an energy and carbon source (Glass and

Orphan 2012), and therefore increases in methanotrophy does not necessarily produce CO₂ in equal amounts.

2.5.2.2 Nitrogen Cycling

Nitrate, runoff, and combined treatments did not significantly differ with the freshwater control for N₂O flux, soil or pore water NO₃⁻, soil extractable NH₄⁺, or pore water NH₄⁺ (for nearly all dates). This suggests nitrogen balance in soil cores was regulated via another pathway not accounted for, possibly reduction of N₂O to N₂ or direct production of N₂ via anammox. Additionally, Cu loading did not appear to induce microbial toxicity for denitrifiers.

2.5.3 Combined Effects of Saltwater Intrusion and Runoff

CO₂ flux was reduced for the combined treatments. Similar patterns of CO₂ flux for combined treatment with individual salinity and copper treatments (Fig. 2.2a) suggest high salinity, specifically high Cl⁻ (Fig. 2.3a), and copper synergistically reduced CO₂ emissions. Although Cu decreased CH₄ flux, the combined treatment did not have a significant effect on CH₄ flux over the experiment. This suggests seawater or nitrate play a role in regulating effects of copper on methanogens or methanotrophs. For the combined treatment, N₂O flux was higher for only one date (Week 4) compared to the pre-treatment flux. Additionally, N₂O and DEA were not significantly different across treatments indicating negligible effects of combined saltwater intrusion and runoff on N₂O or the potential of denitrification.

In our study, the effects of saltwater intrusion and runoff on carbon and nitrogen cycling are limited to soil microbial processes, but hydrology and plants also play a major role in regulating biogeochemical transformations in soils. Drought conditions and fluctuating hydrology regulate nitrogen and carbon cycling in wetlands. In coastal plain wetlands, combined effects of soil drying and saltwater intrusion reduced dissolved organic carbon (DOC) to a

greater extent than drying or saltwater intrusion alone (Ardón et al. 2013) suggesting a greater decrease of DOC flux when salt water intrusion occurs during periods of drought. Although denitrification is generally higher in areas of fluctuating water levels due to coupled nitrification-denitrification (Hernandez and Mitsch 2007), CH₄ generally decreases with fluctuating water levels (Itoh et al. 2007; Altor and Mitsch 2008).

Additionally, plant interactions with carbon and nitrogen cycling vary greatly depending on plant species and physiochemical environment. Across a salinity gradient, plant species composition ranges from freshwater species to saltwater tolerant species, but increasing salinity for freshwater plants lead to plant mortality (Crain et al. 2004) and shifts in community composition (Glenn et al. 1995). Differences in plant communities regulation of carbon and nitrogen cycling is supported by differences of CO₂ and CH₄ emissions, rhizospheric oxidation, acetate decomposition depending on the plant species (Strom et al. 2005). Additionally, increased nitrogen demand of *Phragmites australis* compared to *Spartina patens* was associated with increased nitrogen mineralization rates (Windham and Meyerson 2003). Denitrification was 300% greater in *Phragmites australis* sediments compared to *Spartina patens* suggesting contrasting effects of plants on nitrogen cycling in wetlands.

Increased runoff and saltwater intrusion to wetland ecosystems will continue to threaten the valuable ecosystem functions wetlands provided. Our study suggests the synergistic reduction of CO₂ flux due to combined effects of saltwater intrusion and runoff are primarily driven by salt (Cl⁻) and Cu toxicity. Similarly CH₄ was significantly inhibited by Cu. N₂O flux increased over time with salinity, although N₂O flux and denitrification potential were not significantly different between treatments over the experiment. Similar patterns of increased N₂O and NH₄⁺ mobilization with salinity are apparent and require further investigation to identify

threshold levels in which salt or sulfate affects different steps of denitrification. A basic understanding of the mechanisms and magnitudes of chemical loading (i.e. metals, salinity, and nutrients) on carbon and nitrogen processes would be the first step to estimating carbon and nitrogen processing responses to anthropogenic stressors at larger scales, although interactions with plants and hydrology also need to be considered.

Table 2.1 Description of experimental treatments and chemical components. AFW = artificial freshwater and ASW = artificial seawater.

Treatment Group	Treatment Name	Components	Grouping	Salinity (ppt)	Justification
Control	Freshwater	Artificial freshwater (AFW)	Control	0.08	Prepared following (Smith et al. 2002)
Saltwater Intrusion	Seawater	Artificial Seawater (ASW)	Saltwater intrusion	18	Assuming upper limit of mesohaline salinity class (Cowardin et al. 1979) Prepared following (Kester et al. 1967)
	Saltwater	ASW no sulfate	Saltwater intrusion	18	K ₂ SO ₄ replaced by KCl
	Sulfate	Sulfate to levels in ASW	Saltwater intrusion	2	Sulfate to levels in ASW
Runoff	Nitrate	AFW + Nitrate	Runoff	~0.08	Nitrate in CT river export and runoff (2 mg L ⁻¹ N as NaNO ₃ ; Pitt 2015; Mullaney 2016)
	Copper	AFW + Copper	Runoff	~0.08	Copper in runoff (100 µg L ⁻¹ Cu as CuCl ₂ ; Odnevall Wallinder et al. 2009; Pitt 2015)
	Runoff	AFW + Nitrate + Copper	Runoff	~0.08	
Saltwater Intrusion + Runoff	Combined	ASW + Nitrate + Copper	Saltwater intrusion + Runoff	18	

All treatments received 4 mg C L⁻¹ as potassium acetate; the average concentrations of total organic carbon in CT rivers (Mullaney 2016)

Table 2.2 Mean (\pm standard deviation) of soil physiochemical properties including organic matter (SOM), extractable ammonium ($\text{NH}_4^+\text{-N}$), extractable chloride (Cl^-) and sulfate (SO_4^{2-}) for the top and bottom soil layer for eight treatments and pre-treatment conditions (Ambient)

	SOM		$\text{NH}_4^+\text{-N}$		Cl^-		SO_4^{2-}	
	%		mg kg^{-1} soil		mg kg^{-1} soil		mg kg^{-1} soil	
	0-5 cm	5-10 cm	0-5 cm	5-10 cm	0-5 cm	5-10 cm	0-5 cm	5-10 cm
Ambient	17.2* (1.1)	16.6* (1.3)	142* (70.2)	106* (37.7)	4714* (1271)	4056* (1788)	1382* (420)	1017* (298)
Freshwater	14.8 ^{abc} (1.0)	14.9 ^b (1.0)	72.8 ^{ab} (49.2)	113 ^{ab} (40.8)	1289 ^c (319)	1853 ^{cd} (278)	2462 ^b (531)	1985 ^{abc} (480)
Sulfate	16.4 ^a (1.4)	17.6 ^a (1.7)	18.2 ^b (15.3)	56.2 ^c (21.6)	1117 ^c (206)	1377 ^d (275)	6263 ^a (1389)	3345 ^a (305)
Saltwater	16.4 ^a (1.3)	16.4 ^{ab} (1.4)	35.3 ^{ab} (5.55)	75.1 ^{abc} (18.9)	19115 ^a (1791)	10678 ^b (1954)	1823 ^b (295)	1399 ^c (446)
Seawater	15.7 ^{ab} (0.6)	15.9 ^{ab} (1.3)	29.5 ^b (12.4)	63.5 ^{bc} (20.6)	11837 ^b (2975)	8334 ^b (2419)	2942 ^b (1272)	2003 ^{abc} (774)
Nitrate	13.8 ^{bc} (1.4)	15.3 ^{ab} (1.0)	100 ^a (57.8)	136 ^a (27.3)	1059 ^c (238)	1848 ^{cd} (309)	2276 ^b (169)	1768 ^{bc} (363)
Copper	13.5 ^{bc} (1.1)	14.8 ^b (1.3)	61.2 ^{ab} (21.7)	105 ^{abc} (30.0)	1444 ^c (429)	2270 ^c (540)	2469 ^b (401)	1732 ^{bc} (208)
Runoff	13.9 ^{bc} (0.7)	14.9 ^b (1.5)	30.0 ^b (18.6)	73.6 ^{bc} (20.3)	1302 ^c (135)	1634 ^{cd} (300)	2223 ^b (677)	2173 ^{abc} (628)
Combined	15.1 ^{abc} (0.6)	16.3 ^{ab} (1.1)	22.3 ^b (16.8)	70.8 ^{bc} (30.8)	23993 ^a (8573)	17288 ^a (3787)	3418 ^b (1615)	2562 ^{ab} (688)

$\text{NH}_4^+\text{-N}$ was square root transformed and Cl^- and SO_4^{2-} were \ln transformed prior to analysis. Letter indicate significant differences among treatments ($p < 0.05$) from ANOVA for each depth interval. * indicates Ambient properties were not including in analysis.

Table 2.3 Results from repeated measures ANOVA comparing the effects of treatments over time for carbon dioxide (ΔCO_2), methane (ΔCH_4), and nitrous oxide ($\Delta\text{N}_2\text{O}$)

	ΔCO_2 Flux			ΔCH_4 Flux			$\Delta\text{N}_2\text{O}$ Flux		
	df	F-value	P	df	F-value	P	df	F-value	P
Date	7	16.9	<0.0001	7	2.11	0.0448	7	10.2	<0.0001
Treatment	7	26.3	<0.0001	7	10.1	<0.0001	7	6.87	0.0001
Date*Treatment	49	3.11	<0.0001	49	1.38	0.0721	49	0.72	0.9072

Table 2.4 Results from repeated measures ANOVA comparing the effects of treatments over time for pore water sulfate (SO_4^{2-}), chloride (Cl^-), ammonium (NH_4^+), and copper (Cu)

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

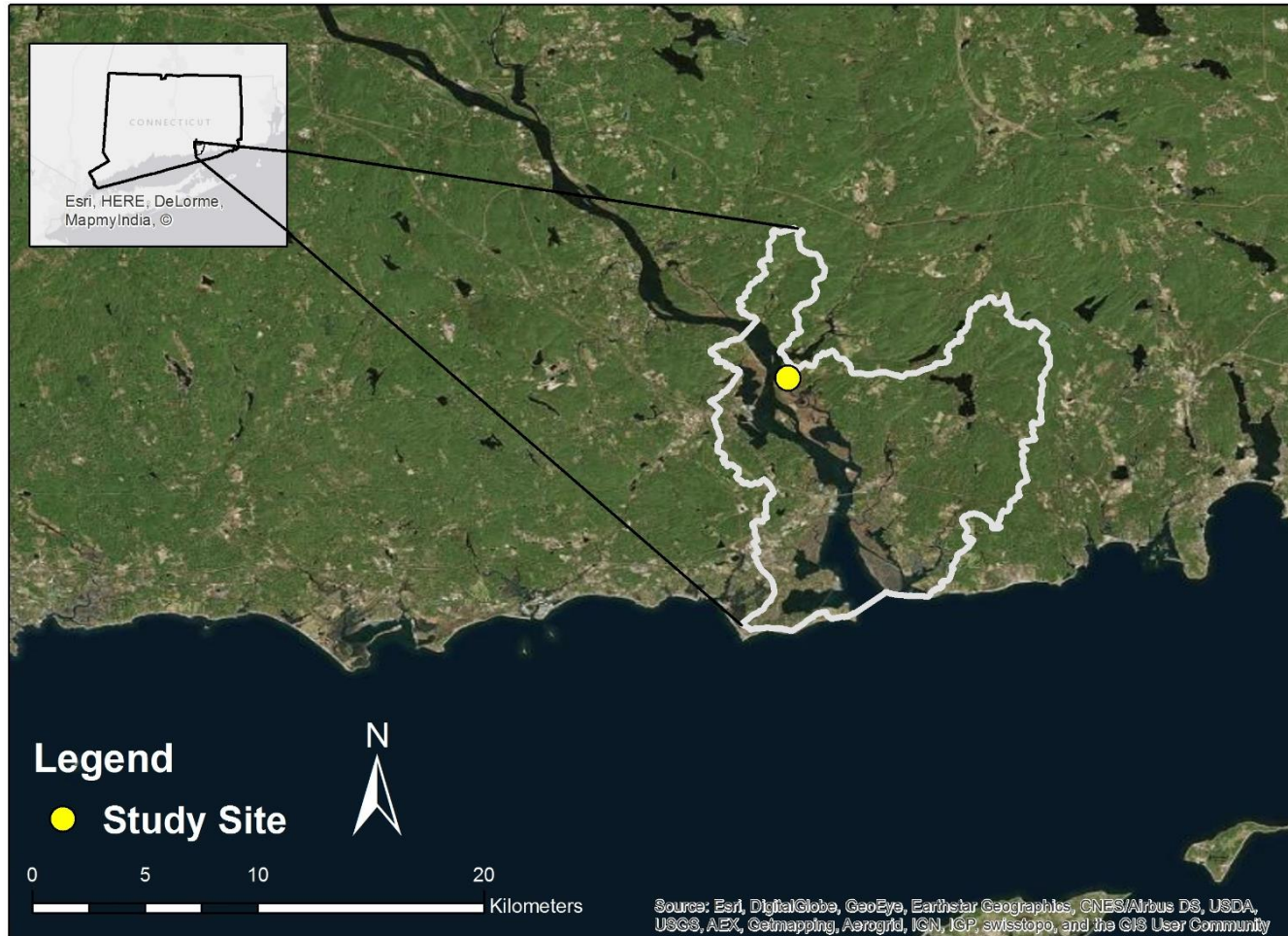


Figure 2.1 Sampling site location (yellow circle) within the HUC-12 watershed CT mainstem - Joshua Creek to mouth (grey outline) (USDA-NRCS 2001)

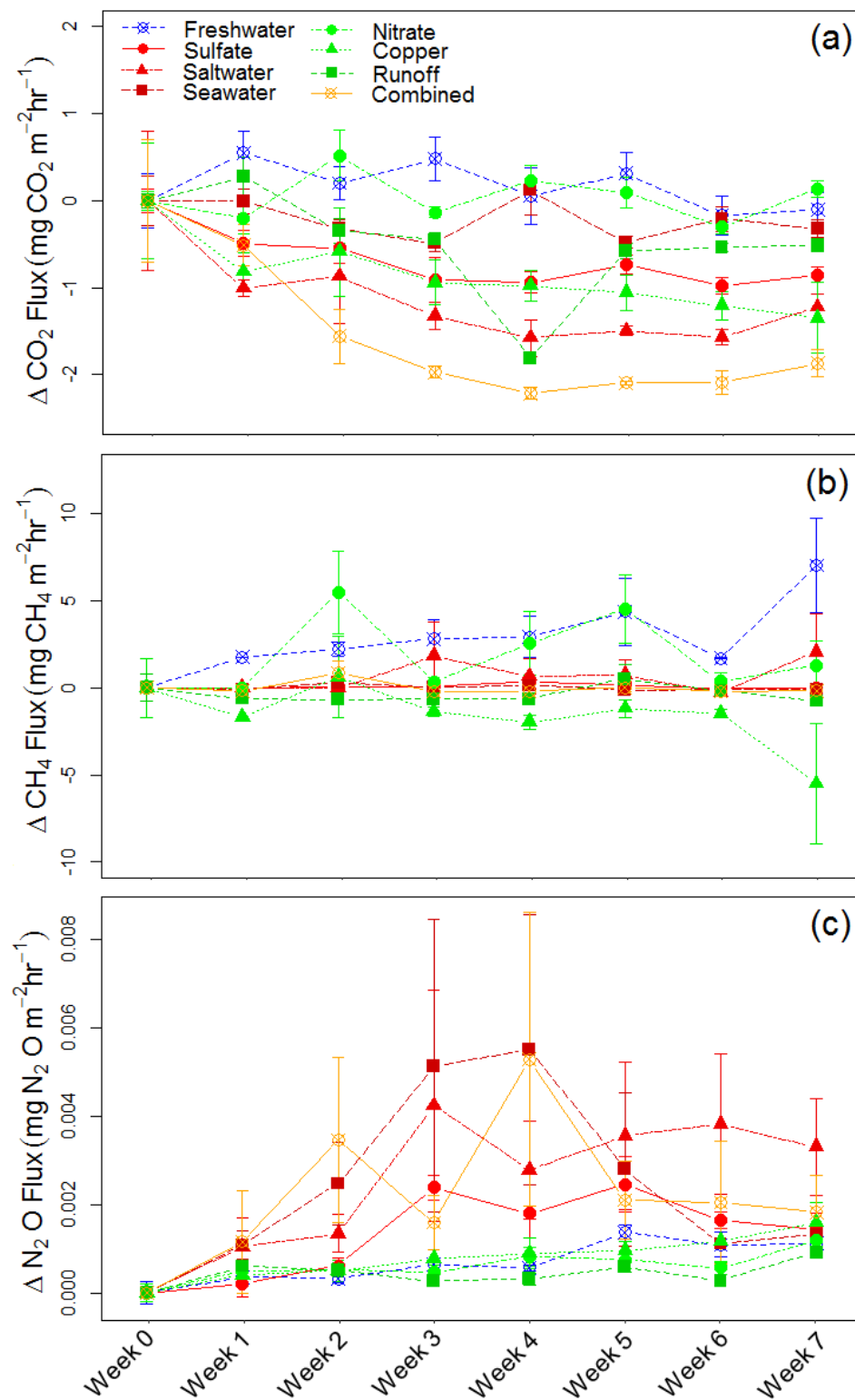


Figure 2.2 Interaction plots of change in greenhouse gas flux (\pm standard error of mean) since pre-treatment flux of carbon dioxide (ΔCO_2), methane (ΔCH_4), and nitrous oxide ($\Delta \text{N}_2\text{O}$) over seven weeks.

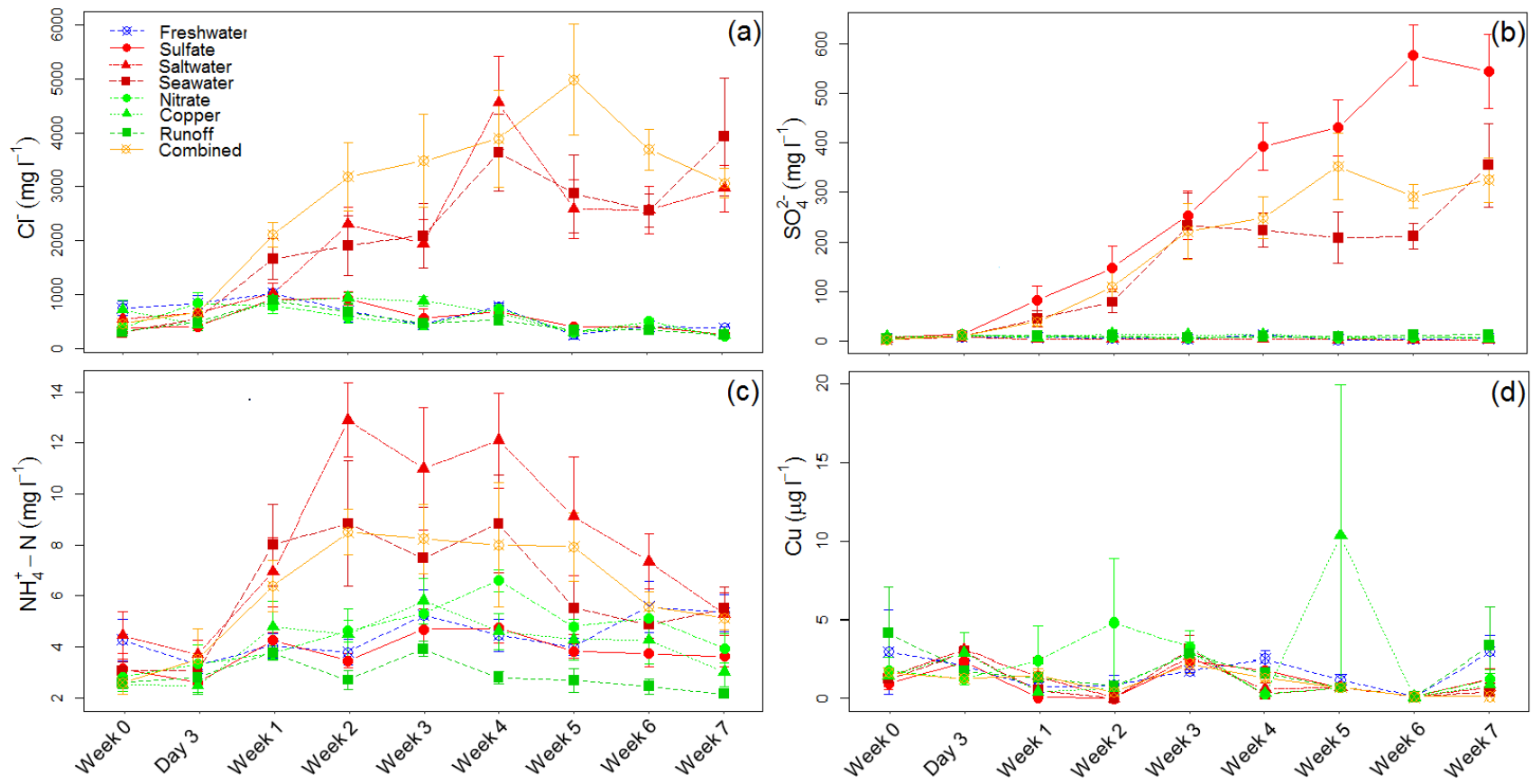


Figure 2.3 Interaction plots of mean pore water concentrations (\pm standard error of mean) of chloride (Cl^-), sulfate (SO_4^{2-}), ammonium (NH_4^+), and copper (Cu) over seven weeks.

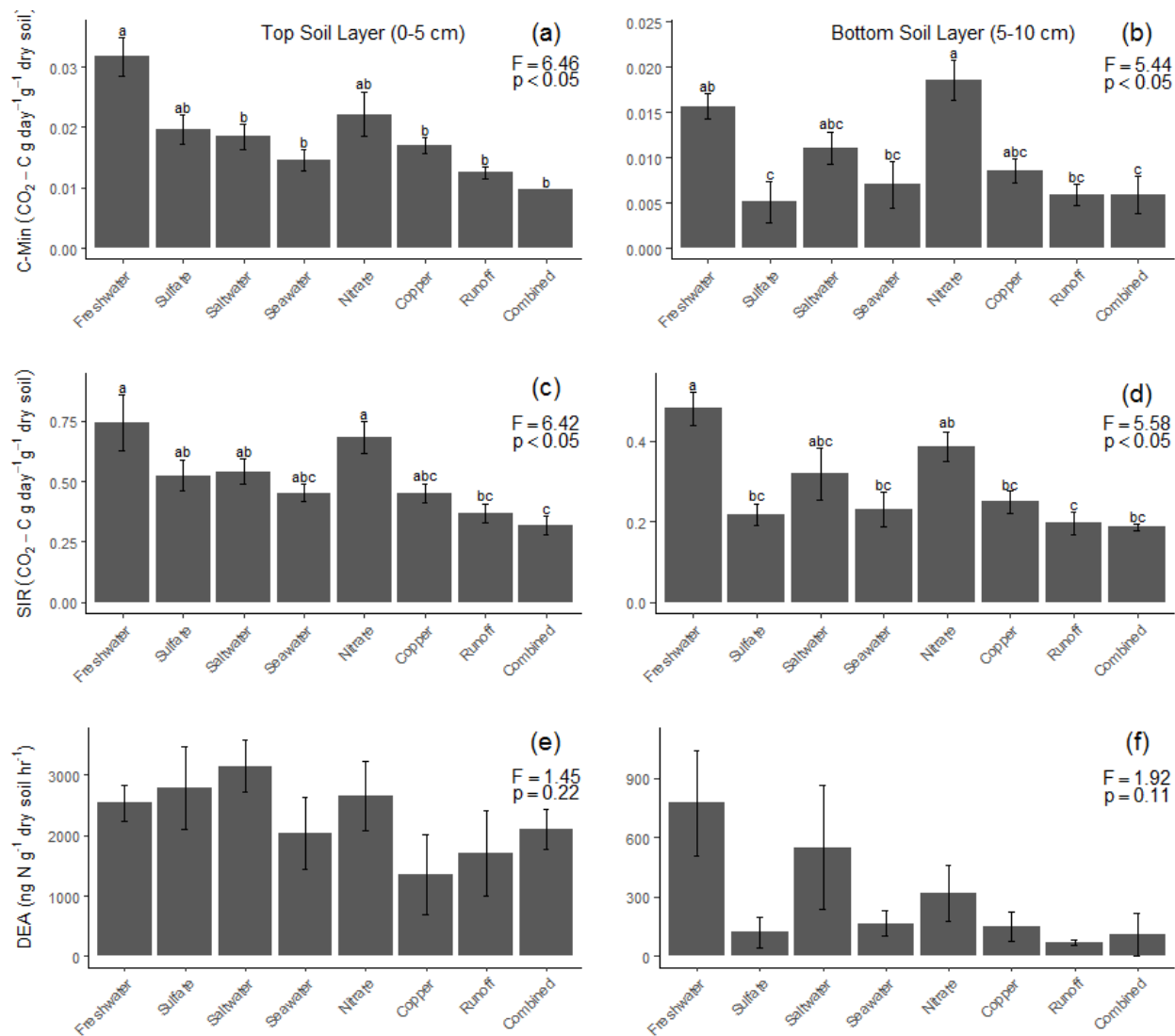


Figure 2.4 Mean rate (\pm standard error of mean) of carbon mineralization (C-Min) for the top soil layer (a) and bottom soil layer (c) and microbial biomass (SIR) for the top soil layer (b) and bottom soil layer (c) for eight treatments. C-Min and SIR were \ln transformed prior to analysis. Letters denote significance ($p < 0.05$).

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