ABSTRACT

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DECIPHERING CORE RECORDS OF
CARBON AND NITROGEN IN TYPHA-
DOMINATED FRESHWATER
WETLANDS

Rumya Ravi, Master of Science, 2022

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I conducted decomposition experiments and examined soil characteristics in restored and natural freshwater marsh platform sites to decipher core records of soil C and N. Carbon loss rates and changes in $\delta^{13}C$ and $\delta^{15}N$ were obtained from decomposition experiments. Core samples at each site were analyzed for bulk density, weight %C, %N, $\delta^{13}C$, and $\delta^{15}N$. *Typha* C loss rates were similar among sites, and there was little change in $\delta^{13}C$ composition, suggesting that DOC leaching is significant. Core carbon storage is higher in natural wetland sites. Initial *Typha* %N and $\delta^{15}N$ reflect local N concentrations and sources to each wetland. $\delta^{15}N$ increases between decomposed vegetation and upper cores in the tidal wetlands, possibly indicating denitrification. In N-rich wetlands, core %N and $\delta^{15}N$ reflect differences in N sources and changes in N sources over time. In a wetland limited by N transport, core %N and $\delta^{15}N$ may reflect vegetation N uptake.

DECIPHERING CORE RECORDS OF CARBON AND NITROGEN IN TYPHA-
DOMINATED FRESHWATER WETLANDS

By

Rumya Ravi

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Chapter 1: Introduction

Soil organic carbon (SOC) is Earth's largest carbon reservoir. Although wetlands occupy 5-8% of land surface area, they store 20-30% of global SOC (Mitsch and Gosselink, 2007). Rates of carbon storage are high (~118 g-C cm^{-2} yr^{-1}; Mitsch et al., 2013), which suggest that wetland restoration could increase C storage. Recent studies of carbon storage in Coastal Plain wetlands (called "blue" carbon in saltwater wetlands; "teal" carbon in freshwater wetlands) and inland wetlands in different climatic regions indicate that in the U.S., Coastal Plain wetlands store more carbon than any other US region, except for cold northern wetlands in the Upper Midwest and Mountain regions (Fig. 1.1; Nahlik and Fennessy, 2016). This suggests that protecting or restoring Coastal Plain wetlands might be an effective target for carbon (and nitrogen) management, but it is important to assess the conditions that promote or limit C storage.
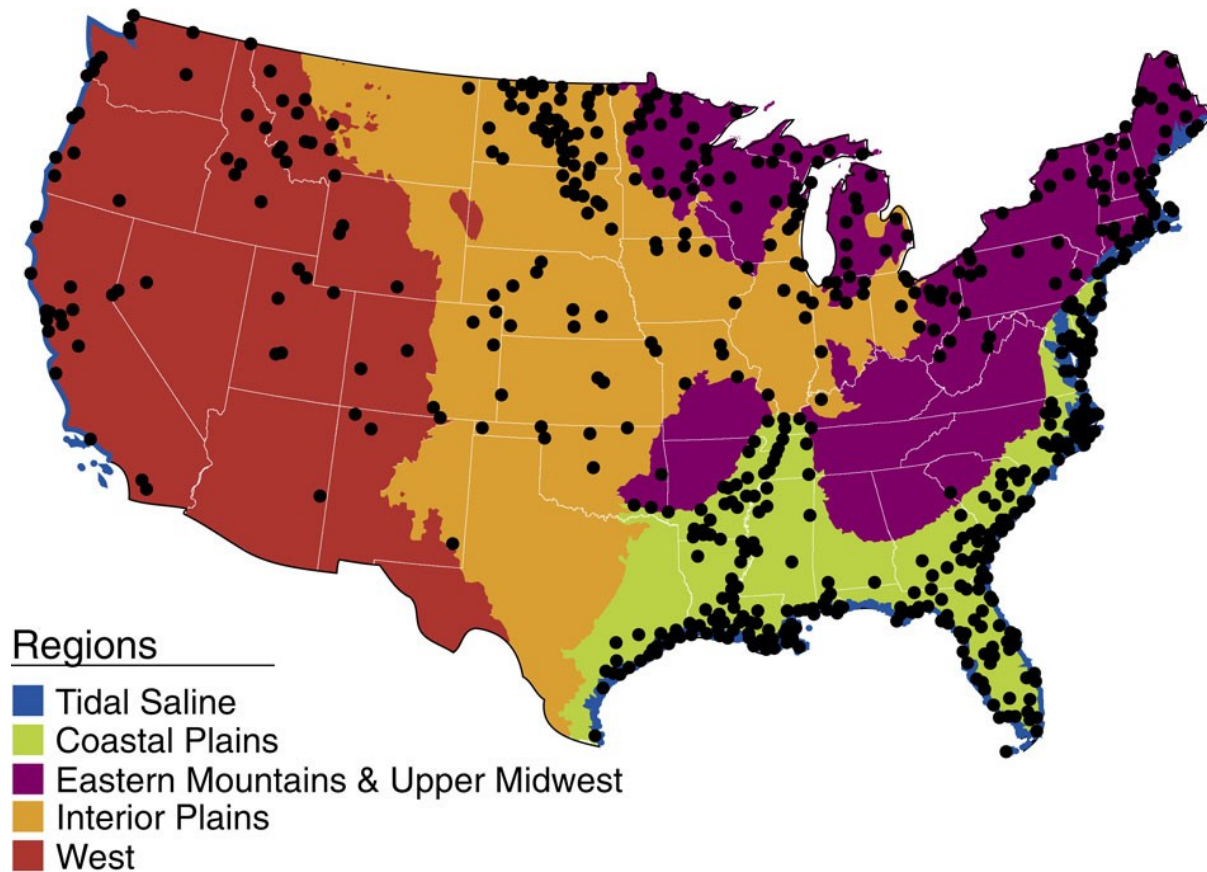
Figure 1.1. Map of the distribution of wetland probability sites. Figure from Nahlik and Fennessy, 2016. Note the high density of points (studied wetlands) in the green Coastal Plains and blue Tidal Saline regions.

Carbon storage in freshwater wetlands involves many fluxes and reservoirs. Reservoirs of carbon in freshwater tidal wetlands include biomass and carbon-rich sediment, which is primarily organic, but can include inorganic sediment (bicarbonate and carbonate minerals). Loss mechanisms of organic carbon from a wetland include decomposition of vegetation and soil organic matter (SOM), dissolved fluxes of organic and inorganic carbon (DOC and DIC), and sediment particle mobilization and removal. Fluxes of carbon away from coastal wetlands are important parts of the global carbon cycle: potentially up to 91% of C export to oceans is DIC, and up to ~80% of DOC in coastal wetlands is exported to the ocean for recycling or further storage and burial (Barrón and Duarte, 2015; Bogard et al., 2020; Cai, 2010). Fluxes of carbon towards and away from wetlands can change storage in wetland reservoirs, so any variables that affect these fluxes can exert control on carbon sequestration in freshwater wetlands. Many of

these variables are influenced by climate (temperature, precipitation, and hydrodynamic processes).

Hydrological processes can exert controls on carbon fluxes and reservoirs. Flow characteristics such as water depth, flow rates and volumes, and air/water temperatures can vary among and within wetlands. For example, freshwater tidal wetlands often exhibit systematic variations in discharge, velocity, and tidal amplitude between tidal inlets and marsh interiors. Inlet water levels reflect local tidal forcing, but tidal amplitudes often decrease up-marsh due to decrease in channel depth and increases in channel roughness. Episodic pulses of high or low flow conditions can be influenced by evapotranspiration and storms. These hydrological processes may affect the duration and frequency of marsh soil saturation, which can affect plant decomposition rates, SOM abundance, species composition and plant health. Wetland temperature is affected by regional climate, but also by sources of water and inundation frequency. Marsh areas adjacent to tidal channels may experience different inundation and drainage dynamics than the high marsh platform at higher elevations or further from tidal channels. Hydrological processes can vary significantly within and among wetlands, so it is important to monitor it as a factor that can affect carbon storage.

Microbial decomposition involves biotic processes that can remove carbon and nitrogen from wetland storage. These processes can often be identified through changes in isotopic composition. Microbial degradation of vegetation and SOM can occur aerobically in the presence of oxygen and anaerobically in anoxic, waterlogged conditions. Bacteria, fungi, and Archaea can metabolize dead plant tissues and SOM, which releases greenhouse gases such as carbon dioxide, methane, or nitrous oxides into the atmosphere. Decomposition rates vary with oxygen availability: anaerobic decomposition is less efficient and releases less mineralized carbon by volume than aerobic respiration (Schädel et al., 2016). Decomposition rates are also

affected by the microbial diversity and vegetation recalcitrance: sugars and starches are less recalcitrant (easier to decompose) than structural compounds like lignin or cellulose (Ma et al., 2018). Microbial decomposition removes carbon from wetland soils; therefore, variations in decomposition rates could impact the amount of carbon stored in a wetland.

Climate change can affect carbon sequestration in freshwater wetlands through changes in temperature, hydrological characteristics, and ecological communities. As sea level rises and the frequency and magnitude of storm events increases, flooding regimes (from higher tides, more frequent and higher storm surges, or increased precipitation events), average water levels, and water level distributions could change. Warming atmosphere temperatures could cause lengthening of the growing season and/or faster organic matter decomposition rates, which could have conflicting impacts on organic matter storage (Morris et al., 2016). In addition, freshwater wetlands must maintain elevation above mean sea level to preserve carbon stocks. For a freshwater tidal marsh to keep pace with climate change and sea level rise, accretion must equal or exceed material losses and sea level rise (Temmerman et al., 2003). At equilibrium:

$$inorganic\ sediment\ +\ organic\ sediment\ = \\ decomposition\ +\ compaction\ +\ sediment\ export\ +\ sea\ level\ rise.$$

In coastal tidal wetlands (both saline and freshwater), organic matter accumulation is a significant component of vertical accretion that maintains marsh elevation as sea-level rises (Nyman et al., 2006). Climate change, however, affects hydrology, temperature, primary productivity, and vegetation composition, all of which can influence organic matter accumulation and decomposition in contradictory and inconsistent ways, and my study attempts to untangle some of these contradictions. For example, sea level rise and subsidence create subtidal space for organic matter accumulation and tidal wetlands. In addition, redox conditions, high salinity, long inundation times, and high water temperatures have each been shown to speed up, slow down, and have no effect on vegetation decomposition (Lalimi et al., 2018; Middleton,

2020; Mueller et al., 2016; RoyChowdhury et al., 2018; Su et al., 2007). Similarly, a marsh with high primary production and dominated by recalcitrant species of vegetation may quickly accrete carbon in sediment, but high biodiversity might also increase decomposition rates and carbon loss (Ibanez et al., 1999; Stagg et al., 2017).

The multiple factors that influence organic carbon accretion and decomposition in a wetland may have competing effects on carbon storage. This study examines *Typha latifolia* decomposition in hydrologically distinct freshwater wetlands, and links carbon retention and composition to the long-term carbon accretion in these wetlands through analysis of wetland sediment core records. Decomposition of surficial vegetation and SOM, particle erosion, and DOC fluxes can each influence the carbon and nitrogen composition of the sediment profile. Therefore, determining plant decomposition rates and isotopic composition and comparing these data to down-core changes in organic matter carbon and nitrogen composition could clarify the roles of aboveground decomposition and hydrological patterns in SOM.

Hydrological and climatic conditions influence biomass accumulation, decomposition rates, and leaching of dissolved components from organic soils. As sea level rises and climatic/hydrological patterns change, ranges of plant species may shift, if there is non-urban space inland to occupy. Therefore, examining the accumulation of organic carbon in wetland reservoirs and the fluxes of carbon away from these reservoirs can help determine whether restored or natural freshwater wetlands can maintain themselves, increase carbon storage in the marshes, and even be part of climate change mitigation. Knowing how the potential for carbon storage could vary with climate change and wetland restoration can help us better understand the fate of restored and natural wetlands.

Previous work on decomposition rates and carbon storage

There are few studies that compare decomposition rates, and isotopic composition of vegetation with characteristics of underlying peat material (via analyses of cores). Even fewer studies monitor physical characteristics of sites (hydrology, temperature) under comparison during decomposition and other studies. Field-based studies employ experimental or observational (e.g., Ribaudo et al., 2012; Palinkas and Koch, 2012; and others) approaches. Experimental studies include mesocosm experiments, such as Mueller et al. (2016), who investigated the effect of flooding and the presence of vegetation on decomposition and SOM. They installed both barren and vegetated mesocosms (of 10 cm diameter each) at different elevations (15 cm below the marsh platform surface, at the platform elevation, and 20 cm above the platform) in a Maryland brackish tidal marsh, and monitored SOM isotopic composition and CO_2 fluxes from the soil to investigate decomposition rates of SOM. This experimental field approach allowed the authors some degree of control over the natural wetland system, while maintaining many of the natural environmental characteristics (within a narrow range of elevations). Another common type of field-based experiments are leaf-pack decomposition experiments (e.g., Stagg et al., 2017) that monitor decomposition by monitoring the loss of weight of leaf or root packs placed in field sites.

Many field studies are primarily observational, often with detailed monitoring of physical, chemical, and biological parameters, or they combine observations with experiments. For example, Stagg et al. (2017) monitored water levels, redox potentials, and catalogued dominant vegetation species during their decomposition experiments. Ribaudo et al. (2012) and Palinkas and Koch (2012), among others, monitored environmental variables like gas fluxes and sediment grain size. Observational studies like these can allow for quantitative records of

environmental changes over multiple timescales, whether daily changes in water levels, changes in sedimentation over years, or seasonal variations in vegetation.

Other studies of carbon content changes and carbon storage in freshwater tidal wetlands use laboratory (e.g., RoyChowdhury et al., 2018) or modelling approaches (e.g., Kirwan and Murray, 2007). Laboratory and modelling approaches can help identify specific variables that may affect carbon storage. These approaches, however, rely on previous results to constrain their models. Without the observation of in situ responses, they may miss important, complex interactions among variables that may affect carbon storage. Some studies (e.g., Stagg et al., 2017) combine modelling and field approaches; they used field data to develop an exponential model for decomposition rates and a Structural Equation Model (SEM) to tease out the environmental variables affecting decomposition rates.

The advantage to combining observational, experimental, and modelling approaches is that multiple types of data can be collected and analyzed to gain a fuller picture of processes that affect carbon storage. A limitation of a combined study is that it requires integrated sampling and monitoring and holding some variables constant (in this case plant species) so that the effects of others can be determined. This study examines carbon storage through field-based experimental and observational methods.

Objectives and hypotheses

Carbon stock comparison in natural and restored wetlands

A purpose of this study is to compare carbon density and carbon stocks among and within natural and constructed freshwater wetlands, both tidal and nontidal. I examine the C, N, and OM throughout sediment cores across the wetlands and determine C and N storage.

I hypothesize that:

1. *Marsh sites with higher peak primary productivity values will sequester more C and N than other sites.*

   An important reservoir of C and N in wetlands is the vegetation itself. A higher primary productivity would drive overall C and N production in a wetland higher. More biomass at the peak growing season also means that there will be more organic material containing C and N that will die off and degrade into the soil at the end of the growing season. In all the marshes, I expect the *Typha* aboveground biomass (g dry weight per m^2) be higher near the inlets due to steady influxes of nutrients and water, and therefore higher C and N sequestration.

2. *Marsh sites with faster Typha leaf and stem decomposition rates will sequester less C and N.*

   Decomposition of organic compounds transforms them into forms of C and N (e.g. CH_4, CO_2, N_2O, dissolved forms of C and N) that typically escape

sequestration. Sites with faster litter decomposition rates should lose more of the organic material in the leaf litter before transformation into soil and compaction.

3. *Sites in established wetlands will sequester more C and N than recently restored or constructed wetlands.*

   An older, less disturbed wetland should have more time to accrete organic material containing C and N than recently constructed wetlands. Recently constructed wetlands have not had time to develop large organic soil horizons.

4. Null hypotheses: Sites with higher peak productivity, sites with faster *Typha* decomposition rates, and site age will not be related to C and N sequestration.

Decomposition experiments

The second purpose of this thesis is to compare decomposition rates, and C, N, and OM dynamics of *Typha latifolia* aboveground biomass as it decomposes across natural and constructed freshwater wetland sites. I will examine whether site environmental parameters: surface, groundwater and air temperatures, groundwater levels, primary production, and vegetation composition affect decomposition rates. At each site, the chemical composition of fresh and decomposed leaf litter will be compared with the chemical composition of the upper part of the soil cores.

I hypothesize that:

1. *Marsh sites with the highest vegetation biodiversity will have the fastest Typha decomposition rates.*

   Organic soil in barren areas with no vegetation does not decompose as fast as soil in planted areas due to the priming effect (Mueller et al., 2016). The priming effect is when a small input of labile organic material to the soil stimulates microbes in the short term to decompose all SOM faster, including older SOM that previously was more recalcitrant (Kuzyakov et al., 2000). Vegetation adds biomass when it senesces, so priming of the microbial community may be more likely in areas where there is high biodiversity.

   Site plant diversity may affect decomposition rates. Litter decomposition rates have been shown to decrease with decreases in plant diversity when there are only one or two functional groups (i.e. growth and N-fixing habit types; Hector et al., 2000). I expect that plant diversity will be higher in the sites in natural wetlands, as opposed to the sites in constructed urban wetlands. Even after years, constructed, disturbed, or urban wetlands still may be primarily dominated by *Typha*, especially since some wetlands are restored with monospecific plantings (Reinartz and Warne, 1993). Newly constructed wetlands may be as diverse as natural wetlands, but after 4-7 years, constructed wetlands decline in diversity (Stefanik and Mitsch, 2012). Because the constructed wetlands in my study are both older than 7 years, I hypothesize that a natural tidal wetland will have a greater plant diversity compared to the constructed wetlands, and therefore faster decomposition rates.

2. *Sites with seasonal to weekly intermittent wetting and drying cycles will exhibit the fastest Typha decomposition rates.*

While aerobic decomposition is typically faster than decomposition in waterlogged, anerobic conditions, decomposition rates actually may be faster in areas with seasonal to weekly wetting and drying cycles (Battle and Golladay, 2001). These cycles may stimulate aerobic microbial activity in moist soils more than a consistently dry aerobic environment (Battle and Golladay, 2001).

3. Null hypotheses: Decomposition rates of *Typha* will not be different from the decomposition rates of other plants in these marshes, *Typha* decomposition will not be different between sites with different vegetation composition, and *Typha* decomposition will not be affected by hydrological patterns.

SOM, C, and N distribution in marsh cores and among study wetlands

A third purpose of this study is to examine sediment core carbon and nitrogen content and isotopic composition across wetland sites and as a function of depth. I will examine how C, N, and OM content and stable isotopic compositions in vegetation and cores record changes in C and N inputs, and processes like decomposition, compaction, denitrification, and nutrient uptake. Studying core records of carbon and nitrogen can help evaluate whether cores preserve carbon and nitrogen sources and/or processing via their isotopic signatures and percent content.

I hypothesize that:

1. *Soil organic matter (SOM) content in the upper layer of the cores will be lower in areas with faster Typha decomposition rates.*
   In sites where decomposition of *Typha* is faster, there should be less organic residue accumulating over time in the soil. Because of this, I hypothesize that these sites will

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be less likely to store carbon in the soil profile than they are to export it through decomposition or DOC removal.

2. *SOM content of the soil will be higher near the surface of the soil. I also hypothesize that the SOM content will increase in recalcitrance with depth in the soil profile and will therefore show higher C:N ratios deeper in the soil.*

Because vegetation dies back at the surface and most root biomass, shoot biomass, and microbial biomass is in the upper 20 centimeters of the soil, I expect that the SOM content will be higher near the surface and will decline with depth in the core. I expect that the matter that is preserved deeper in the core will be less labile. Organic matter that is less labile may have a higher C:N ratio because it consists of structural, carbon-rich compounds like lignin and cellulose, so I hypothesize that the C:N ratio will increase with depth in the core as recalcitrant matter remains and is buried in the soil.

3. *C isotopic signatures near the surface of the soil profile will be similar to decomposed vegetation values but will change with depth. I also hypothesize that the ^{13}C content of soil from the sites with more diverse vegetation will be different from the ^{13}C content of soil from the most homogenous sites.*

I hypothesize that changes in the isotopic composition in the upper portions of cores would reflect microbial decomposition, which could be a significant factor affecting carbon storage below the marsh surface. Plant species diversity may correlate with higher microbial diversity (Kim et al., 2022), and enzymatic pathways and cell physiologies are different among different types of microbes. Therefore, I

hypothesize that sites with more diverse plant communities will show a different ^{13}C signature from the homogenous *Typha* communities.

4. *Core C content will reflect the effects of water fluxes at each site. Removal of DOC should cause a decrease in carbon content with relatively little change in carbon isotopic composition.*

   Water can physically remove and transport away particles, but it can also leach dissolved carbon and nitrogen away from marsh soils (Álvarez and Bécares, 2006). Since leaching removes C already in a dissolved form without microbial transformation, it should decrease core C with little fractionation of isotopes.

5. Null hypotheses: Core carbon content and isotopic composition will be similar between all sites and will be similar at all depths of cores. The core carbon content and isotopic composition will have no correlation with surface decomposition rates, amount of tidal flushing, primary productivity, or vegetation type.

Roadmap of thesis

Chapter 2 describes the characteristics of the three study wetlands. I also describe the field instrumentation and other field methods used to conduct this study, including field litter decomposition experiments, biomass and vegetation surveys, and hydrological monitoring. I will discuss methods used for vegetation and core chemical analysis, including bulk density and % OM analyses, % C and % N analyses, and isotopic analyses.

The results of the study are presented in Chapters 3 and 4. Chapter 3 will present the results of the analyses of the vegetation field experiments. It outlines the results of the biodiversity and biomass surveys at each wetland site. I will detail the results of the initial C content, $\delta^{13}C$, N content, and $\delta^{15}N$ composition of *Typha latifolia* aboveground biomass across the three wetlands. Then I will present the results of the litter decomposition experiments to evaluate C and N loss rates and changes in isotopic composition during decomposition and compare the chemical compositions of the vegetation to the upper layers of soil cores as litter is transformed into organic soil.

Chapter 4 compares core data across three wetland sites. It starts by discussing the bulk density, OM, and % C data, and uses the results of that to calculate the soil carbon storage at different wetland sites. I will then discuss the nitrogen data (% N, $\delta^{15}N$, and C:N ratio) at each site and calculate N storage as well. For both C and N core data, I will present both across-wetland comparisons as well as down-core data for each site separately. Chapter 5 will conclude and discuss the overall implications of my findings.

Chapter 2: Study sites, methods, and environmental characteristics

Study wetlands

The three study wetlands are within 30 km of each other and have similar climatic regimes (Fig. 2.2, Fig. 2.3). The 30-year mean annual temperature for the region is 13.8 °C, and the 30-year mean annual precipitation is 115 cm (2021 PRISM dataset). Sites are all freshwater wetlands and sampled locations are high marsh platforms dominated by *Typha latifolia.* Tidal wetlands and flow-through wetlands have channels that deliver water to the wetlands. Often, especially in tidal wetlands, there is a lower platform directly above the channel that is inundated frequently and at an intermediate depth (0 – 0.8 m depth, mean 0.6 m; Statkiewicz, 2014). This lower platform is dominated by emergent vegetation that thrives when partially submerged at specific depths (i.e. *Nuphar lutea* in the Patuxent River wetland; Fowler, 2014, Statkiewicz, 2014). Above the lower platform, there is an upper platform that is inundated even less frequently and at a shallower level, and is dominated by plants such as *Zizania aquatica* (0 – 0.7 m depth, mean 0.45 m; Statkiewicz, 2014). Further up the platform, the marsh is only inundated between mean high tides and the highest spring tides and is dominated by a wide variety of plants, notably cattails (*Typha latifolia*), but also sedges (*Carex* sp.), Virginia cutgrass (*Leersia virginica*), marsh sunflowers (*Aster* sp.), jewelweed (*Impatiens capensis*), and other species. High marsh platforms will get inundated during spring tides, but unless subject to an extreme storm event as well (e.g. tropical storms), the water may not have sufficient energy to bring sediment with it to accrete on the platform surface (Baranes et al., 2022; Goodwin and Mudd, 2019).

The first wetland site studied is the natural freshwater tidal wetland (24.7 Ha), located on the tidal Patuxent River, Maryland. Inundation is caused by diurnal tides, with overbank flooding associated with spring tides. Less frequent overbank flooding is also caused by river flooding. River flood events associated with storm events generate discrete episodes of high turbidity (suspended sediment). Some of this sediment is deposited on vegetation. Patuxent River total nitrogen inputs have been high compared to the other sites (~ 1.7×10^6 kg/yr; Boynton et al., 2008) but have been declining in recent years due to improved wastewater treatment. Nitrogen inputs are from atmospheric deposition, fertilizer runoff, and sewage from upstream sites (Seldomridge and Prestegaard, 2014).

The urban restored freshwater tidal wetland (4.0 Ha) is located near Bladensburg, Maryland in the upper Anacostia River estuary. The wetland was constructed in 2010 by dredging sediment from the river bottom and constructing a marsh platform with a gravel substrate upon which marsh vegetation has grown and accreted organic matter. The marsh organic soils are about 30 cm thick. The urban tidal wetland experiences overbank inundation with both tides and storm events. Adjacent urban watersheds generate large and fast ("flashy") spikes in water levels in the tidal river and in the marsh. Recent (2003-2011) nitrogen inputs to this wetland are from atmospheric deposition, fertilizer, and sewage runoff (~ 98,200 kg/yr; Miller et al., 2013). Nitrogen loads are not as high as the historic N loads in the Patuxent River estuary (difference of 1.6×10^6 kg/yr) .

The urban nontidal wetland (1.9 Ha) is located in the UMD Golf Course in College Park, Maryland. This wetland was accidentally constructed in 2001 when an earthen dam failed and turned a pond into a wetland. Water inputs are driven by storm events on two headwater streams with some input from urban runoff in the headwaters. The water input dynamics are extremely "flashy", but stormflows move quickly through the wetland as overland or partially channelized

flow. Baseflow discharge, however, moves through the wetland primarily as groundwater flow. Nitrogen inputs are from fertilizer and atmospheric deposition, which has been declining (NTN Station MD13). Fertilizer runoff from the golf course is well managed, and primarily contributes N via groundwater to stream baseflow (concentrations ~2-4 mg/L; Prestegaard, pers. comm.). Storm N loads are more dilute, but can deliver significant quantities, especially during post-storm baseflow, a dynamic observed in other coastal plain streams (O'Connell, 1998). Stormflow incoming and outcoming peak discharges are similar, suggesting that stormflow moves quickly through the wetland. Baseflow in shallow groundwater moves through the wetland at rates of 10 – 30 m/day, based on the hydraulic conductivity, gradient, and porosity of the wetland sediments (Prestegaard, pers. comm.). Therefore, groundwater flow can take several weeks to traverse the wetland, providing ample opportunity for plant uptake, dilution by infiltrating precipitation, and other processes, to affect pore water chemistry.
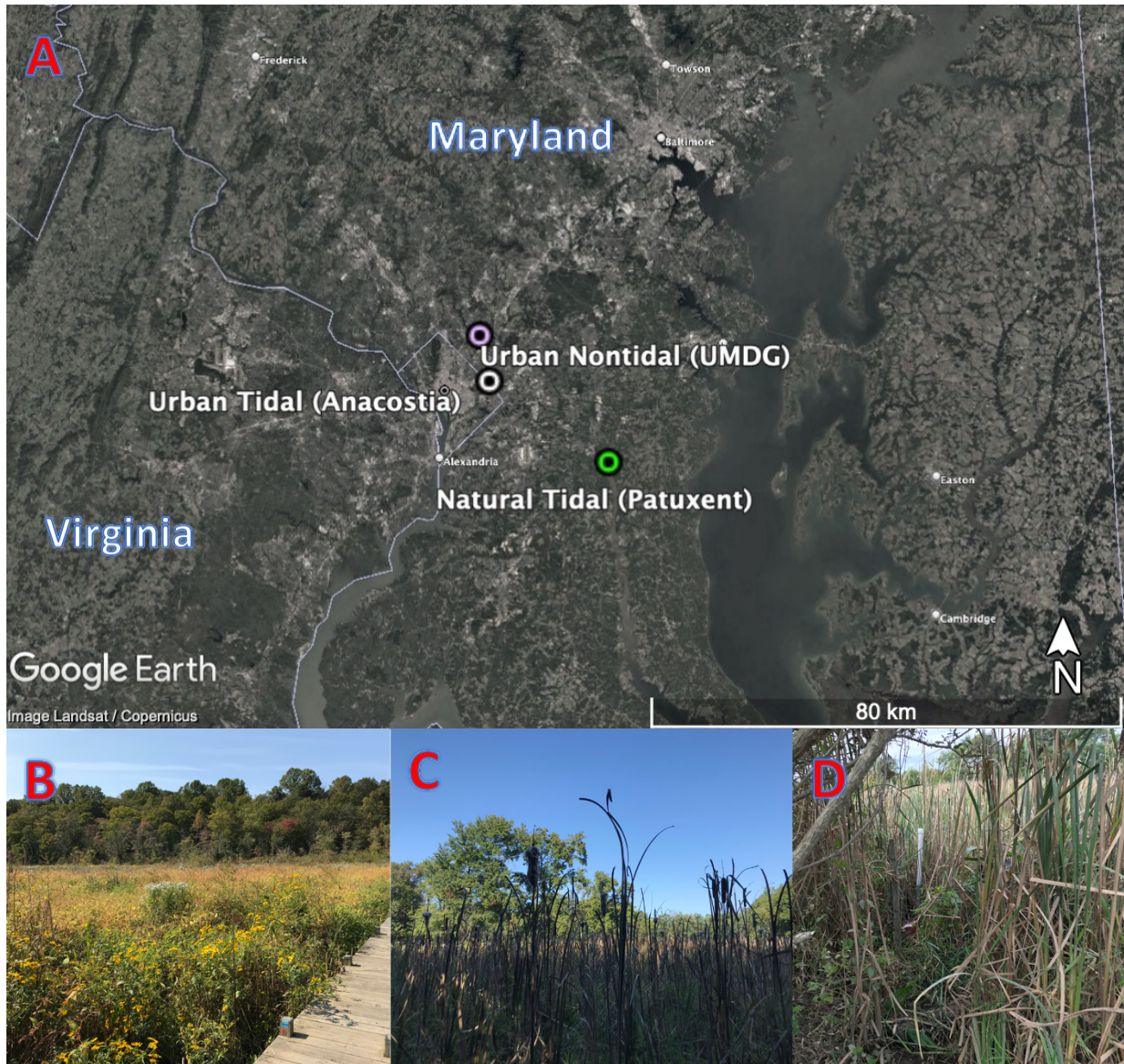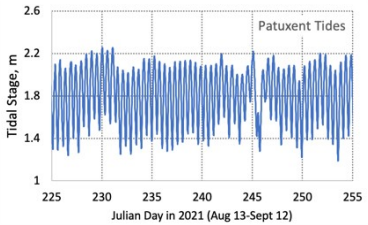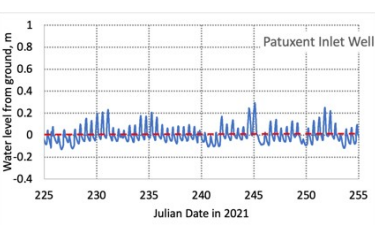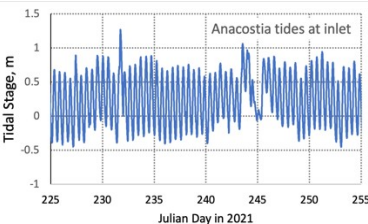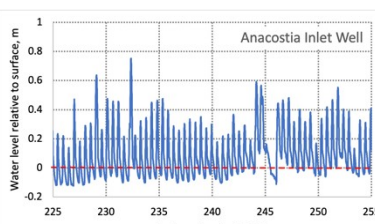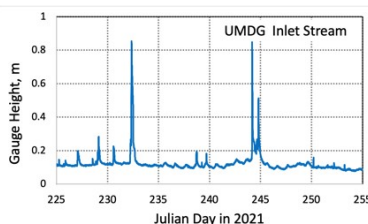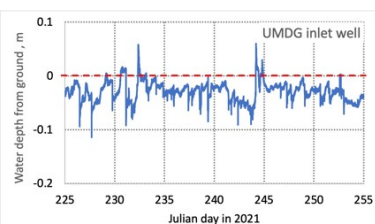
Figure 2.1. Map and photographs of study sites. (A) Map of the three study sites in Maryland, all within 30 km of each other. (B) September 2020 photograph of interior site at natural tidal wetland (Jug Bay, Patuxent River). (C) September 2020 photograph of interior site at urban nontidal wetland (UMD Golf Course). (D) September 2020 photograph of inlet site at urban tidal wetland (Bladensburg, Anacostia River).

Table 2.1. Summary of study wetland characteristics.

Site	Images of wetlands	Water sources	Time series of input water levels	Time series of inlet well levels relative to surface	N sources	N load
Natural tidal (Patuxent) - 38º48'07.01" N 76º42'20.40" W		Tidal			Atmospheric deposition Sewage Fertilizer runoff	High
Urban tidal (Anacostia) - 38º55'28.09" N 76º56'11.23" W - Constructed 2010		Tidal precipitation			Atmospheric deposition Sewage Fertilizer runoff	Med
Urban nontidal (UMD Golf Course) - 38º59'44.93" N 76º57'18.28" W - Constructed 2001		Stream precipitation			Atmospheric deposition fertilizer runoff	Low

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Hydrological conditions at the three sites

The three sites are in the same climatic region and although the same storm may not affect each site, similar storm events do. The Coastal Plain of Maryland has a humid, temperate climate with hot summers and cold winters. There is considerable inter-annual variation in precipitation. Conditions for the field experiment study period (September 2020 – February 2021) are summarized below (Fig. 2.2 and Fig. 2.3).
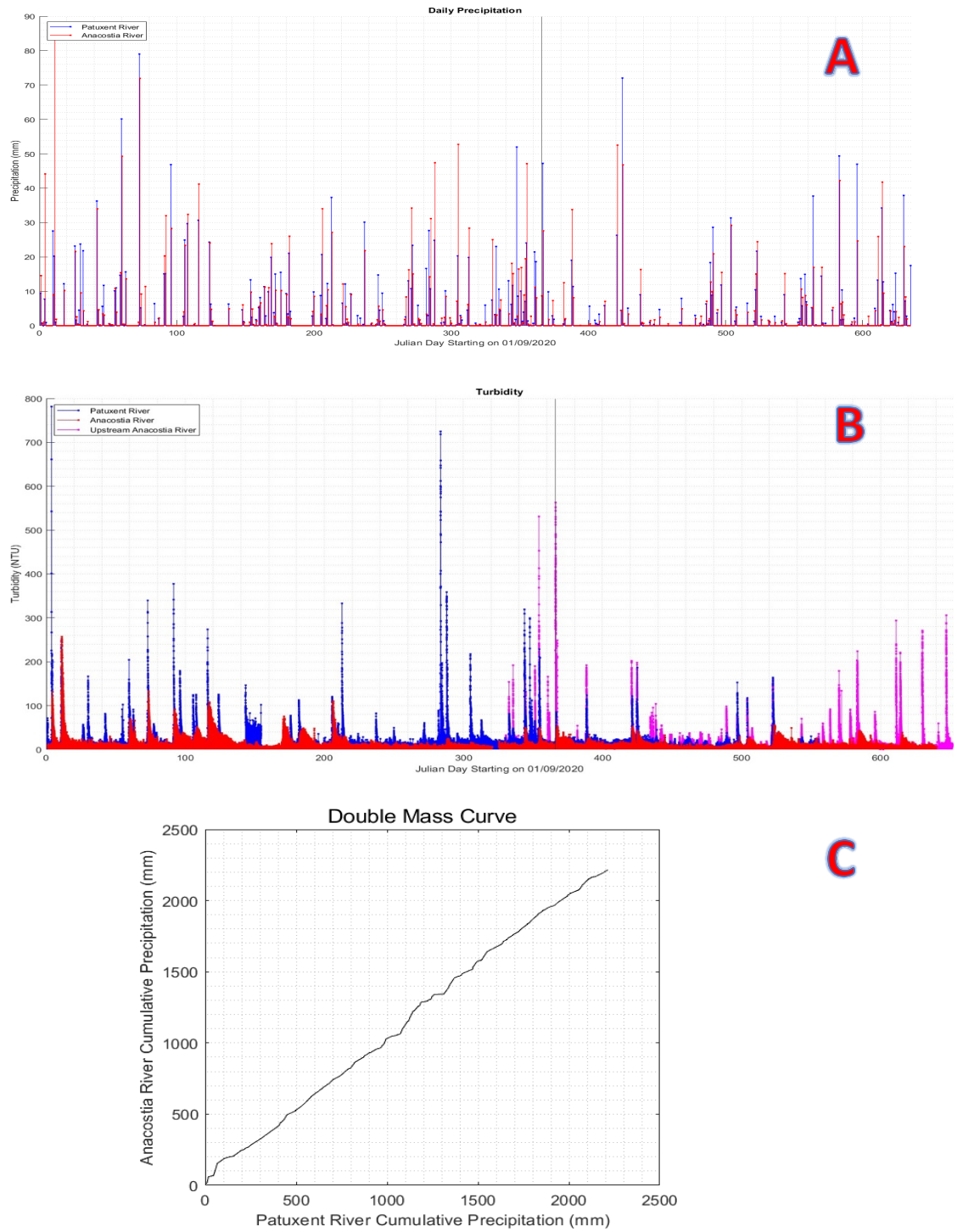
Figure 2.2. Precipitation and turbidity at study sites. Daily precipitation (A) and turbidity (B) for the lower Anacostia River Basin (near both urban wetlands) and the Patuxent River (natural tidal wetland). Date starts on September 1, 2020. (C) Double mass curve, indicating an almost 1:1 relationship in the cumulative precipitation for the Anacostia and Patuxent sites. Figure constructed by Alex Lastner based on PRISM data.
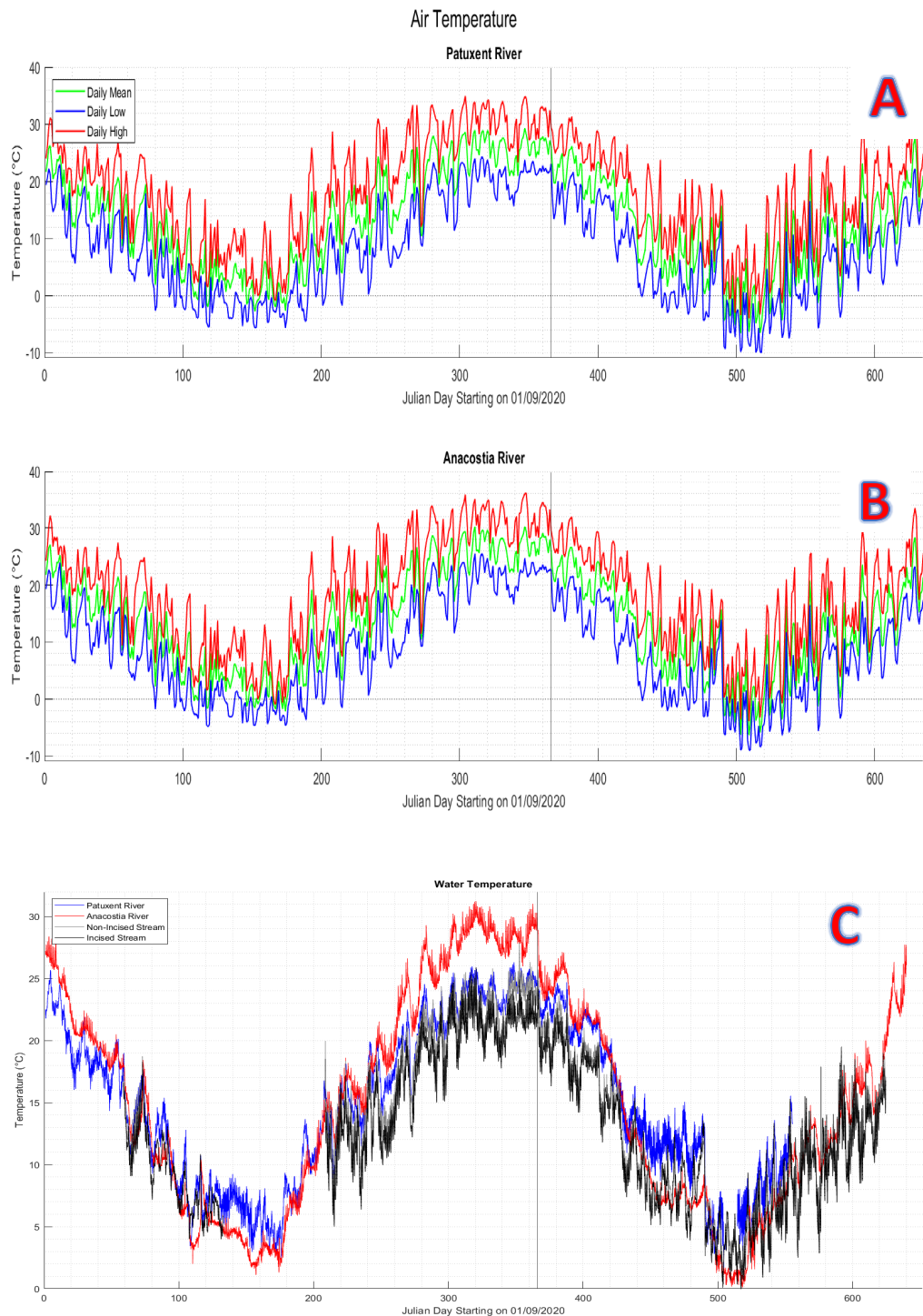
Figure 2.3. Air and water temperatures near study wetland inlets. Air temperature (max, ave, min) for the Patuxent River (A), air temperature, (max, ave, min) for the Anacostia River (B), (C) wetland inlet water temperature for Patuxent, Anacostia, and creek inlets to the non-tidal wetland. Figure constructed by Alex Lastner based on PRISM data (air temperature), USGS, and Eyes on the Bay data (water temperature).

Field methods

Aboveground biomass, stem diameter and height, and vegetation diversity

Typha aboveground biomass was determined by sampling in three 1 m^2 quadrats at each core site location. Biomass was collected at each site growing season in September – October 2020. In three 1 m^2 quadrats at each site, stem height and diameter were measured for every species at three points during the growing season in 2021 (June, July, and end of August). In quadrats where there were more than 10 stems of a species growing, all stems were counted, but a random 10 were measured for height and diameter and average height and diameter were calculated. In 2021, at three points during the growing season, species richness at each wetland was determined by counting the total number of species found across the sites in each wetland. During the peak of the growing season (July), the Simpson's Diversity Index for each site was calculated for the vegetation:

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

where n is the number of individuals of a species, and N is the total number of individuals in the sample (Simpson, 1949). Species richness indicates the number of species in a plot; species evenness indicates how evenly distributed different species are in a plot. The index includes both richness and evenness but weights evenness more heavily (Nagendra, 2002). As a few very rare species growing in a wetland are less likely to affect carbon and nitrogen in soil than evenly distributed species, I will be using the Simpson's Diversity Index to measure diversity.

Leaf litter carbon and nitrogen loss experiments

Decomposition experiments were conducted at seven marsh platform sites across the three

freshwaterwetlands, with 2-3 sites at each wetland. In each wetland, at least one site was located

near the inlet and one in the interior of the marsh. These sites were chosen to provide a range of

hydrological settings. In September 2020, five mesh litter packs (each with approximate volume

of 0.25 ft^3 and mesh size of 5 mm) of *Typha* aboveground biomass (leaf, stems, and fruiting

bodies) were installed at each of the seven sites (Fig. 2.4). The *Typha* in each pack was collected

at each site itself; each pack included a proportion of leaves, stems, and fruiting bodies that

roughly represented proportions of those parts on living plants. Each pack was then dried and

weighed before deploying. The *Typha* packs were collected at designated time intervals,

scheduled based on the assumption of an exponential decay rate (similar to Fowler, 2014). After

retrieval of a pack, the *Typha* was dried at 60 °C for 24 hours and weighed to determine mass

loss. Subsamples of the dried material were analyzed to find percent organic matter and percent

carbon. OM content was determined by loss on ignition via combusting subsamples at 500 °C for

4 hours (e.g. Su et al., 2007; Álvarez and Bécares, 2006; and Jackson et al., 1995) and weighing

the remaining ash. It is assumed that processing the subsamples this way combusts all organic

matter, leaving behind inorganic material. Once the inorganic fraction was determined, the

equation:

$$\% \, OM = \left(1 - \frac{inorganic \, LOI \, subsample \, weight}{initial \, subsample \, weight}\right) * 100\%$$

was used to calculate percent or fraction OM.

Although many previous studies examine decomposition rates by evaluating mass loss

(Álvarez and Bécares, 2006; Battle and Golladay, 2001; Fowler, 2014; Hector et al., 2000;

Ibañez et al., 1999; Lan et al., 2006; Longhi et al., 2008; Su et al., 2007; Tang et al., 2020; Zhu et al., 2021) I found it difficult to remove fine clay associated with frequent river flooding and algal films from plant matter. Therefore, decomposition rates were assessed as the change in organic matter fraction and C fraction over time (e.g. Ryder and Horowitz, 1995). These methods are reviewed below.

Laboratory methods

Core sampling and analysis

Two to four cores were obtained from each wetland, with at least one core at each of the 7 decomposition experiment sites (Fig. 2.4). Cores were up to 50 cm in length and were frozen until analyzed. Cores were inspected to determine organic matter depth. Organic-rich soil extended to 30 cm in the urban, constructed tidal wetland and 40 cm in the constructed nontidal wetland (with organic-rich pond sediment below 40 cm). The natural tidal wetland was organic to depths greater than 50 cm. Cores were sectioned into $10 - 11$ increments (each $2.5 - 5$ cm in length), thawed, and oven dried at 60 ºC for 24 hours. Core sections (wet and dried) were weighed, and dry bulk density was calculated as:

$$Dry\ Bulk\ Density = \frac{Dry\ weight}{Volume\ of\ core\ section}$$

The core section volume was calculated as:

$$Core\ section\ volume = \frac{(core\ radius^2 * \pi * core\ section\ length)}{2}$$

The core radius for the core sampler used is 2.38 cm. The % organic matter was determined by LOI as described above. Dried subsamples of core sections were analyzed in an elemental

analyzer with mass spectrometer to determine both the C and N content and their stable isotopic signatures using the procedures outlined below.

Analyses of carbon, nitrogen, and their isotopic compositions in vegetation and core samples

C and N concentrations and isotopic analyses were obtained from subsets of dried *Typha* aboveground biomass and core samples. These subsamples were prepared for analysis by powdering them with an agate mortar and pestle and placing up to 20 mg of powder into a tin capsule. C and N composition and isotopic analysis were conducted with a Euro EA elemental analyzer in line with an Elementar Isoprime mass spectrometer. $\delta^{13}C$ were calculated as:

$$\partial^{13}C = \left(\frac{R_{sample}}{R_{PDB}} - 1\right) * 1000,$$

and $\delta^{15}N$ were calculated as:

$$\partial^{15}N = \left(\frac{R_{sample}}{R_{Air}} - 1\right) * 1000,$$

as in Liu et al. (2021) and Zhang et al. (2018), and reported per mil (‰).

As in Hansen and Sommer (2007), the elemental analyzer with gas chromatograph and mass spectrometer was calibrated by repeatedly (twice every 10 samples) measuring an internal lab standard with both C and N (urea) and a reference organic soil sample with known C and N percent composition and isotopic values (LECO 309 with % C of 8.49% ± 0.35% C and % N of 0.73% ± 0.01% N for vegetation samples and LECO 062 with % C of 2.05% ± 0.12% C and % N of 0.19% ± 0.01% N for core samples) to monitor instrument drift and calculate uncertainties. The standard deviations of the repeated LECO percent C and N and references was used as the uncertainties for the study samples $\delta^{13}C$ and $\delta^{15}N$.

Figure 2.4. Photographs of core and *Typha* litter packs. (A) Photo of a core collected at the natural tidal inlet. (B) Photo of a HOBO water level recorder at the nontidal interior site with litter packs deployed for the *Typha* decomposition experiments.

Chapter 3: Biomass, Biodiversity, and C and N Characteristics of Natural and Urban

Freshwater Wetland Vegetation

Results

Biomass and vegetation diversity

The aboveground characteristics of *Typha* in the three wetlands are summarized in Table 3.1 and

Table 3.2. The two constructed urban wetlands are dominated by *Typha*. The natural tidal

wetland contains a zone of *Typha* along the main inlet channel, which is the area of the wetland

sampled for this study. The natural tidal wetland contains a high total biomass at the inlet (760.9

g), but overall has a low *Typha* stem density (1.3 stems per m^2 in the interior). The *Typha* in the

natural tidal wetland also is shorter in the interior (141.6 cm). The Simpson's diversity index for

vegetation was higher in this marsh channel edge interior sites (0.74) than the other wetland sites

examined (diversity indices from 0.30 – 0.58).

The urban tidal wetland contains more aboveground biomass per area in the interior

location than in the inlet (714.6 g and 295.1 g respectively). This wetland also has a low species

diversity (diversity indices of 0.56 and 0.30 for the inlet and interior respectively) and a very

high *Typha* stem density (the interior had the highest *Typha* density of all sites, 10.0 stems per

m^2). The urban tidal inlet site has a lower *Typha* stem density (7.3 stems per m^2) than the interior

site – it is more diverse but sparsely vegetated.

The urban nontidal wetland has a high biomass per area of total aboveground vegetation

compared to other sites, with the highest values at inlet and middle sites (923.4 g and 889.5 g,

respectively). However, there is a higher *Typha* stem density in the interior site (6.7 stems per

m^2). The diversity data show a higher species diversity in the nontidal wetlands' interior (index

of 0.58) than in the inlet or middle (indices of 0.51 and 0.42 respectively). In the inlet, the

Typha is fairly tall compared to the other wetlands and sites (190.0 cm), while the interior *Typha*

stems are more numerous but shorter (stem density of 6.7 stems per m^2 and average height of

181.2 cm).

Table 3.1. End of 2021 growing season (August 17-24) aboveground biomass and *Typha* stem density and heights.

Wetland	Site	Biomass per area (g per m^2)	*Typha* stem diameter (cm)	*Typha* stem density (stems/m^2)	Avg. *Typha* stem height (cm)	Simpson's Diversity Index[a]
Natural tidal	Inlet	760.9 ± 0.3	-	-	-	-
	Interior	484.0 ± 0.3	2.3 ± 0.3	1.3 ± 1.2	141.6 ± 27.0	0.74
Urban tidal	Inlet	295.1 ± 0.3	2.5 ± 1.0	7.3 ± 3.8	212.5 ± 53.1	0.56
	Interior	714.6 ± 0.3	2.1 ± 0.7	10.0 ± 4.6	162.1 ± 50.9	0.30
Urban nontidal	Inlet	923.4 ± 0.3	4.2 ± 1.4	3.7 ± 0.6	190.0 ± 27.1	0.51
	Mid	889.5 ± 0.3	4.4 ± 1.8	5.0 ± 1.0	217.5 ± 29.8	0.42
	Interior	481.4 ± 0.3	2.7 ± 1.0	6.7 ± 3.2	181.2 ± 33.0	0.58

[a]Calculated at peak of growing season (July 2021) to get an estimate of peak diversity.

Initial composition of* Typha *leaf and stem material

Carbon content, nitrogen content, C:N ratios, $\delta^{13}C$ values, and $\delta^{15}N$ values in fresh *Typha*

aboveground biomass (leaves, stems, and fruiting bodies homogenized) for sites in the three wetlands

are shown in Table 3.2. Carbon content, nitrogen content, and $\delta^{15}N$ values are higher in the natural

tidal wetland compared to both constructed urban wetlands. The biomass carbon isotopic

composition is similar across all wetlands (~ 29‰). $\delta^{15}N$ values for the natural site were significantly

higher than both urban sites, and the urban nontidal wetland interior's *Typha* has a significantly lower

δ^{15}N value (4.59‰) than the other urban sites. The natural tidal wetland's *Typha* C:N ratio is significantly higher (ratio of 39.59) than the ratios from the urban wetland sites (ratios from 24.11 – 38.48). The natural wetland's *Typha* has more C and N than the urban wetlands' *Typha*, but proportionally more C than N, though that N is isotopically distinct.

Table 3.2. Initial composition of *Typha* leaf and stem material.

Site	% C	% N	C:N	δ^{13}C (‰)	δ^{15}N (‰)
Natural tidal inlet	47.90 ± 0.25	1.21 ± 0.01	39.59	-28.55 ± 0.08	10.24 ± 0.25
Natural tidal interior	47.90 ± 0.25	1.21 ± 0.01	39.59	-28.55 ± 0.08	10.24 ± 0.25
Urban tidal inlet	33.19 ± 0.35	0.93 ± 0.01	35.63	-29.76 ± 0.11	5.30 ± 0.25
Urban tidal interior	36.00 ± 0.35	1.02 ± 0.01	35.43	-28.89 ± 0.11	5.13 ± 0.25
Urban nontidal inlet	41.38 ± 0.35	*	*	-29.29 ± 0.11	*
Urban nontidal mid	34.14 ± 0.35	1.02 ± 0.01	38.48	-30.16 ± 0.11	5.52 ± 0.25
Urban nontidal interior	25.89 ± 0.35	1.07 ± 0.01	24.11	-29.74 ± 0.11	4.59 ± 0.25

* Missing data due to very high uncertainties in replicate %N and δ^{15}N measurements in urban tidal inlet samples.

Composition of upper marsh cores

Carbon content, nitrogen content, C:N ratios, δ^{13}C and δ^{15}N values of the upper 5 cm of the soil cores is shown in Table 3.3. The carbon percentage in the top 5 cm of cores is significantly lower than the initial fresh vegetation values. Upper core carbon content is the highest in the natural tidal wetland sites (14.21% C) and lowest in the urban nontidal wetland (5.58 – 7.65% C). While there is not a strong correlation between initial vegetation and upper core percent carbon, the difference in carbon content between the initial vegetation samples and upper core samples correlates with initial vegetation carbon content and indicates that approximately 75% of the

carbon is lost in the transformation of fresh vegetation to material found in the upper 5 cm of core:

$$\% \ Carbon \ Lost \ = \ 0.76 \ (Initial \ Vegetation \ Carbon);$$

$$R^2 \ = \ 0.79$$

The upper 5 cm of soil cores show a lower percent carbon (~ 10% in upper cores) than the *Typha* litter does after 9 weeks of decomposition, suggesting that full decomposition and storage in soil will take a minimum of 18 weeks if it follows the average decomposition trend line:

$$\% \ C \ = \ -1.56 \ (weeks \ elapsed) \ + \ 36.61.$$

The isotopic composition of the carbon in the upper 5 cm of soil cores is slightly more enriched in ^{13}C across the urban wetland sites (δ^{13}C range of -26.82‰ – -29.07‰) compared to the composition of the decomposed vegetation (δ^{13}C range of -29.29‰ – -30.16‰). The core sample from the natural tidal inlet has a much lower δ^{13}C (-41.74‰) and is excluded as an outlier due to probable contamination. The sample from the natural tidal interior has a similar isotopic composition to the rest of the vegetation samples, indicating no carbon fractionation at that site. In the urban wetlands, although vegetation δ^{13}C does not change significantly during the decomposition experiments, there is a slight enrichment in δ^{13}C between the 9-week decomposed litter and the upper core section (Fig. 3.1 B), which implies that either carbon fractionation occurs as leaf material is added to soil or a material more enriched (such as carbonate) in ^{13}C is being added to the marsh soil but not to the vegetation.

Table 3.3. Composition of upper 5 cm of soil cores

Site	% C	% N	C/N	δ^{13}C (‰)	δ^{15}N
Natural tidal inlet	14.21 ± 0.25	0.93 ± 0.01	15.20	-41.74 ± 0.28	11.56 ± 0.10
Natural tidal interior	11.68 ± 0.25	1.01 ± 0.01	11.61	-29.57 ± 0.33	12.18 ± 0.10
Urban tidal inlet	6.09 ± 0.25	0.47 ± 0.01	13.02	-27.90 ± 0.01	7.03 ± 0.02
Urban tidal mid	6.52 ± 0.35	0.50 ± 0.01	13.16	-28.87 ± 0.33	5.76 ± 0.10
Urban tidal interior	7.71 ± 0.35	0.60 ± 0.01	12.79	-28.83 ± 0.33	7.40 ± 0.10
Urban nontidal inlet	5.58 ± 0.35	0.38 ± 0.01	14.63	-26.82 ± 0.33	3.57 ± 0.19
Urban nontidal interior	7.65 ± 0.35	0.53 ± 0.01	14.52	-29.07 ± 0.33	4.11 ± 0.19

Rates of carbon and nitrogen loss (decomposition experiments)

There were significant variations in weight percent carbon loss in the Typha leaf packs over the course of the decomposition experiments within each wetland, with neither inlet sites nor interior sites showing consistently faster C loss rates. In addition, the interior nontidal site exhibited an increase in % C over time, due to either contamination from grass clippings, fallen tree leaves, algal films, or C-rich sediment that were not properly washed off the leaf packs. Because of the high likelihood of sediment and biofilm contamination of the leaf packs, C loss data were combined across each wetland and averaged across all wetlands and sites to distinguish overall trends in C loss.

Over the course of the decomposition experiments, the weight percent carbon decreases in the *Typha* leaf packs. The average carbon loss decomposition trend is shown above. In the natural wetland, the decrease in carbon may be faster than in the other wetlands (decrease of 3.11% C per week compared to decreases of 1.01% and 1.37% C per week; Fig. 3.1). However, the large uncertainty in these rates (R^2 of 0.33 and 0.20 for the urban tidal and

nontidal wetlands respectively) indicates that the slightly higher rate of C loss in the natural tidal wetland may not be significant. Nevertheless, in all three wetlands, the isotopic composition of carbon does not significantly change as the carbon is lost throughout the decomposition experiments.

As % C decreases in all sites, the inflow water temperature at all sites is also decreasing (Fig. 2.3). The coldest temperature of the period occurs in February, which coincides with the end of the decomposition experiments. Inflow water temperatures briefly may go just below the freezing point 0 ^{o}C (Fig. 2.3).
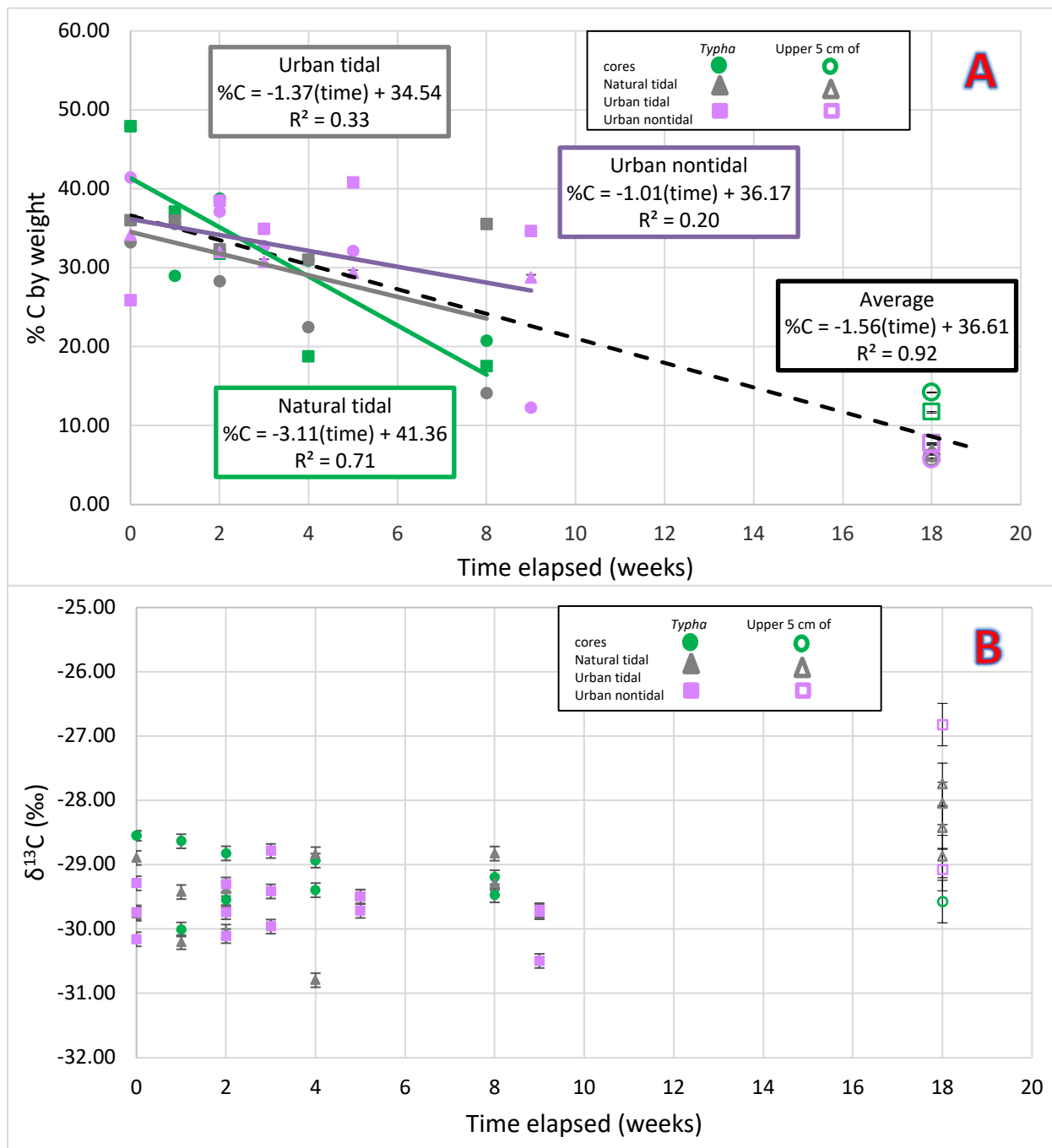
Figure 3.1. *Typha* weight %C and δ¹³C over decomposition experiments. Change in % C (A) and δ¹³C (B) in *Typha* litter decomposition experiments over time. % C and δ¹³C values for surface soil core samples are given as well. Based on trendline for % C loss over time, it is estimated that vegetation will incorporate into upper cores at 18 weeks.

The weight percent of nitrogen in the *Typha* aboveground leaf packs changes differently in each wetland over the period of the decomposition study. In the natural tidal wetland, the weight percent nitrogen decreases after one week and then gradually increases over the next seven weeks as carbon is lost preferentially to nitrogen, increasing the weight percent nitrogen. In the urban tidal wetland, nitrogen content increases significantly once the decomposition experiment starts, then decreases after eight weeks. In the nontidal wetland, *Typha* percent N decreases slightly over the experiment time period (Fig. 3.2). Compared to the carbon content in the leaf packs, the nitrogen content in the leaf packs does not decrease as consistently, implying that carbon is lost during the initial stages of decomposition while nitrogen is retained.

The weight percent of nitrogen is predictably lower in the core samples over all sites than in the decomposing vegetation, indicating that nitrogen is in fact lost by the time *Typha* leaf litter decays into the upper layer of soil (Fig. 3.2). However, the percentage of nitrogen loss is lower than that of C loss. Upper core nitrogen ranges from 50 – 83% of the initial vegetation N values. As a result, the C:N ratio is 35.47 ± 5.87 in the initial vegetation and 13.46 ± 1.17 in the upper cores, indicating a proportionally greater loss of carbon.

The natural tidal upper 5 cm core samples are significantly higher in nitrogen content than the urban core samples (0.93 – 1.01% N in natural tidal wetland, 0.38 – 0.60% N in urban wetlands). The natural tidal wetland *Typha* shows a consistently more enriched $\delta^{15}N$ signature over the decomposition experiments.

The urban wetlands' *Typha* nitrogen isotopes are more difficult to distinguish from each other; however, the urban wetlands' upper core sections are significantly different from each other. The urban tidal wetland's upper cores show a more enriched $\delta^{15}N$ signature (5.76 – 7.40‰) than the nontidal wetland's upper cores (3.57 – 4.11‰).

In general, there are no significant and consistent differences in *Typha* % N, C:N ratio, or δ^{15}N between sites in the same wetland, apart from the inlet/middle and interior in the nontidal wetland. The interior *Typha* consistently has lower % N, a more depleted δ^{15}N, and a higher C:N ratio than the *Typha* in the inlet and middle sites throughout the decomposition experiment.

Figure 3.2. *Typha* **weight % N, δ15N, and C:N ratio over decomposition experiments.** Change in % N (A), δ15N (B), and C:N ratio (C) in *Typha* litter decomposition experiments over time. % N and δ15N values for surface soil core samples are given as well. Based on trendline for % C loss over time, it is estimated that vegetation will incorporate into upper cores at 18 weeks.
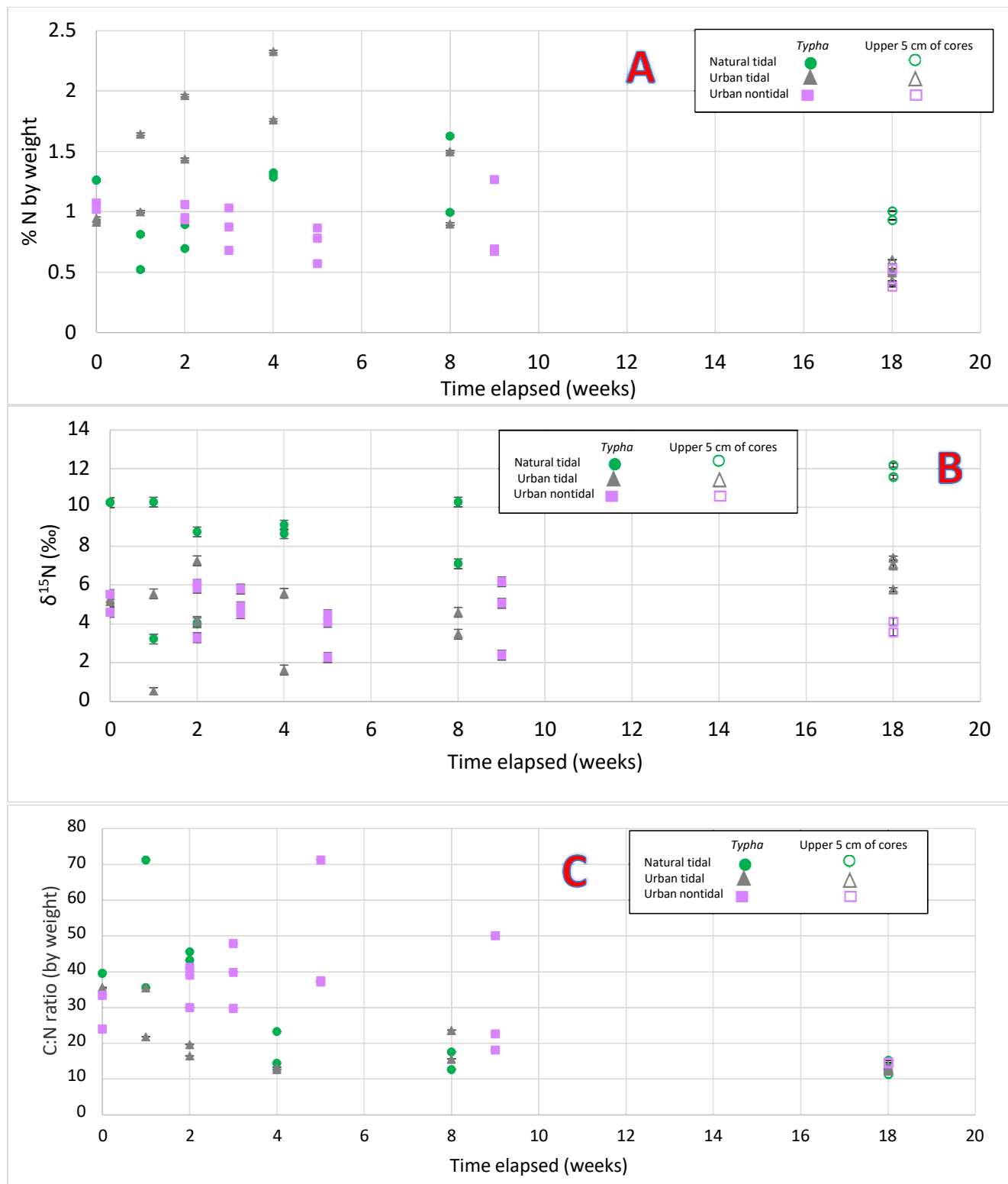
Discussion

Biomass and vegetation diversity

The natural tidal wetland is more diverse in plant species composition than the restored urban wetlands (diversity index 0.74 in natural sites compared to indices from 0.30 – 0.58; Table 3.1). This is somewhat consistent with prior work. For example, constructed wetlands sometimes show more plant diversity than undisturbed wetlands (up to 2 times the species richness), although a higher proportion of this diversity is due to invasive species (Leck and Leck, 2005).

Conversely, while urban wetlands may have the ability to support high diversity, they often do not, as frequent disturbance leaves them open to colonization by aggressive plants like *Typha* sp., which can crowd out other species and decrease diversity (Boers et al., 2007). The natural tidal wetland interior site has a high biodiversity and is not completely dominated by *Typha*, which suggests that it has had time to establish a community before *Typha* could invade. The urban wetlands have lower diversity, especially the interior of the urban tidal wetland (diversity index of 0.30; Table 3.1). As *Typha* reproduces mostly through propagation from rhizomes, it can outcompete other plants an area if the other plants cannot effectively disperse their seeds to the area; *Typha* also tends to dominate in areas that are flooded for more than 35 consecutive days (Boers et al., 2007). Therefore, the urban tidal interior likely is flooded for long durations and does not receive as much transport of native seeds compared to the other wetland sites.

Carbon

As there is a high uncertainty in the carbon loss leaf pack experiments because of problems

washing sediment and algal films off leaf packs, the differences between the C content and

composition of the fresh *Typha* and the upper layer of the cores more reasonably demonstrates

the changes that take place between vegetation die-back and incorporation into soil.

The *Typha* aboveground biomass that starts with a higher carbon content (such as in the

natural tidal wetland) loses a higher percent of carbon as it decomposes (e.g. the natural tidal

inlet *Typha* loses 33.69% C, while the urban tidal inlet loses 27.10% C; Tables 3.2 and 3.3). This

suggests that a higher fraction of the initial carbon in the natural tidal *Typha* was more labile.

Typically, plant leaves contain proportionally more non-structural carbon (starches, proteins,

etc.) than stems do (Ma et al., 2018). These non-structural compounds (sugars, starches, etc.) are

produced through photosynthesis and can be used as energy sources or cell osmotic regulators

for plants; soluble sugars tend to be proportionally stored more in leaves in areas of low water

stress (e.g. wetlands; Jiao et al., 2020). If plant leaves tend to have proportionally more soluble

material in less water-stressed environments, and overall, there is more carbon in the natural

tidal wetland's *Typha*, then it follows that the natural tidal wetland might be a less water-

stressed environment than the constructed wetlands and therefore has a higher proportion of

soluble, labile carbon. Therefore, a higher percentage of that carbon will be removed from the

vegetation as it decomposes into the upper layer of soil.

Leaching or tidal flushing (in tidal wetlands) usually is one of the first processes to

rapidly remove labile, soluble carbon from leaf litter (Álvarez and Bécares, 2006; Ibanez et al.,

1999). The first compounds to leach out of decomposing vegetation must be soluble and are

often leached out very quickly (in a day to a week; Davis et al., 2006). These compounds are

large sources of dissolved organic carbon (DOC) to watersheds. Sugars and metabolites of

sugars (glucose, myo-inositol, etc.) are leached first and in higher quantities, followed by fatty acids (Franklin et al., 2020). Leaching of DOC does not fractionate carbon because it does not involve chemical or microbial transformations of C: therefore, as $\delta^{13}C$ values of the decomposing *Typha* do not significantly change with time at any site (Fig. 3.1), this suggests that the main process removing carbon from the leaf litter is one that does not fractionate carbon.

In addition, carbon is on average lost from the vegetation linearly at all wetlands as water and air temperatures (in the urban nontidal wetland) decrease constantly from throughout the decomposition experiments (Fig. 3.1). Most chemical reactions proceed at faster rates with higher temperatures. Therefore, it follows that the method of carbon loss in the decomposition experiments is not sensitive to temperature. Leaching of dissolved organic carbon (DOC) at least partially involves microbial transformations of organic carbon, which are dependent on temperature, but primarily the carbon loss is dependent on water fluxes rather than temperature (Hagedorn et al., 2010). Accordingly, over the fall and winter period of the decomposition experiments where temperatures are low and evapotranspiration rates are decreasing, carbon leaching may be dominant over microbial decomposition as a carbon removal process in the study wetlands.

Although leaching may be the dominant carbon-removal process in the first 8 – 9 weeks of decomposition, by the time the vegetation incorporates itself into the soil, enough microbial decomposition may have occurred to fractionate carbon isotopes in the upper soil cores. The result is a slightly heavier isotopic signature in the upper cores left behind compared to the decomposed vegetation. Both urban wetlands have a heavier isotopic signature in upper cores compared to the 8- and 9-week decomposing vegetation (Fig. 3.1). Organic material enriched in ^{13}C could have been added to the upper parts of the urban wetlands' cores; or microbial

decomposition could have occurred as vegetation incorporates into the cores. The constructed

wetlands are both located in urban areas; the urban tidal wetland is located next to a cement

plant (~ 125 meters NE from the interior site). The dust from cement, construction zones, and

buildings may be rich in carbonates and may contribute to the enrichment of the upper parts of

the cores in the constructed wetlands. Carbonate-bearing parent materials are common in urban

areas, and carbonate additions like cement dust, concrete waste, and limestone gravel are also

common sources of inorganic C in urban areas that are not present in rural, undisturbed areas

(Lorenz and Lal, 2015). This sediment could be windblown; prevailing winds in the region blow

S-NE throughout the year (Stratton), but even if wind cannot transport the dust to the wetlands,

runoff after precipitation events could carry dust to the interior site as the cement site is at a

higher elevation than the wetland (difference of 6 m). Both lithogenic carbonates and carbonates

formed in soil tend to have less negative $\delta^{13}C$ values (~ 0‰ and ~ -25‰, respectively:

Washbourne et al., 2012) compared to the vegetation leaf packs in this study (~ -29‰). The $\delta^{13}C$

enrichment of the upper cores compared to the decomposing leaf packs in the urban wetlands

may be due to the contamination of isotopically enriched carbonate dust from the urban

surroundings. Even if dust did not directly contaminate the wetland soil, carbonate in runoff

could increase the pH of the soil pore water, which could increase microbial activity in soils,

promoting microbial decomposition of SOM into CO_2 and low molecular weight, labile

compounds that can be leached away from the wetland (Ahmad et al., 2015). The C left over in

the soil may be enriched in ^{13}C, as it is the residue of microbial transformations.

Nitrogen

The natural tidal wetland aboveground *Typha* initially has a higher N content (1.21% N, Table 3.2). This could be because the natural tidal wetland is located on the Patuxent River, which has a higher nitrogen load (1.7×10^6 kg/yr; Boynton et al., 2008) coming from septic, atmospheric, and fertilizer runoff sources than the other sites. The high nitrogen load means the marsh platform vegetation growth is not limited by nitrogen, and therefore the *Typha* can take up ample amounts of nitrogen.

However, the high nitrogen content in the natural tidal *Typha* might decrease over the past years' growing seasons. The Patuxent River has been seeing the effects of pollution reduction efforts: nitrogen loads to the River are classified as "improving" in the long term (since 1985) and in the short term (since 2011) by the USGS (Mason, 2021), slightly decreasing the nitrogen content in the vegetation.

The $\delta^{15}N$ of the initial fresh *Typha* in the three wetlands still appears to reflect major sources of nitrogen (Table 3.2). The more enriched $\delta^{15}N$ composition in the natural tidal wetland (-10.24‰) is likely due to the high loading from septic sources upstream along the Patuxent River, compared to atmospheric deposition and fertilizer sources ($\delta^{15}N$ of 10 – 20‰; Valiela et al., 2000). The more depleted $\delta^{15}N$ composition in the constructed urban wetlands means that they are getting more of their nitrogen from the atmosphere ($\delta^{15}N$ of 0‰) and/or fertilizer ($\delta^{15}N$ of -3 to +3‰; Valiela et al., 2000).

The $\delta^{15}N$ for the *Typha* in all the wetlands remains relatively consistent, showing no fractionation of N during leaf litter decomposition (Fig. 3.2). The *Typha* $\delta^{15}N$ is more enriched in the natural tidal wetland than in the urban wetland. As N in the leaf packs is not lost consistently during decomposition but C is, labile C-rich compounds are leached out of the

Typha leaf litter in the initial stages of decomposition, while N-rich compounds remain.

The $\delta^{15}N$ signatures of the upper cores are significantly different from wetland to wetland (Fig. 3.2). The upper core of the natural tidal wetland reflects the enriched isotopic signature of the nitrogen sources contributing to the Patuxent River system, as observed in the vegetation. The urban wetlands (especially the nontidal wetland) both exhibit lower $\delta^{15}N$ signatures than the natural tidal wetland. This likely reflects the nitrogen sources. Wastewater with more enriched $\delta^{15}N$ could be delivered to the urban tidal wetland through combined sewer overflows in the lower Anacostia River. Denitrification on the marsh platform in the tidal wetlands could also lead to the observed differences in isotopic composition. Nevertheless, the $\delta^{15}N$ signatures of the upper cores of the urban tidal wetland are very similar to the $\delta^{15}N$ signatures of the water in the Anacostia River (~ 5.75‰ to 7‰; Prestegaard and Volz, 2022), which implies that the N from the river sources could be the primary determinant of the $\delta^{15}N$ in the wetland sediment. The differences in isotopic signature between the two urban wetlands are not apparent in the decomposing *Typha* and are only apparent in the upper sediment cores. This may be due to changes in nitrogen loads (decrease of -22.3% per acre) from improved wastewater management in the Anacostia River over the past 10 years (reduction in total N loads by 22.3%; Moyer and Blomquist, 2020), to denitrification occurring only after all labile carbon and nitrogen have been leached out of the vegetation, or both.

The more enriched $\delta^{15}N$ (11.56‰ – 12.18‰) and lower percent N (0.93% – 1.01%) of the two tidal wetlands' upper cores compared to the 8- and 9-week vegetation ($\delta^{15}N$ of 7.1‰ – 10.28‰, % N of 1.00% - 1.63%; Fig. 3.2) implies that either the vegetation and cores are reflecting changes in N sources over the recent few growing seasons, or that denitrification of the vegetation is occurring after 9 weeks of decomposition in just the tidal wetlands. The nontidal wetland upper cores have $\delta^{15}N$ values (3.57‰ – 4.11‰) within the range of the values of the

decomposed vegetation at the same site (2.39‰ – 6.17‰). In the nontidal wetland, denitrification of the decomposed vegetation is likely not removing N based on the similar N isotopic signatures. Interestingly, denitrification may be more likely to occur in areas with low vegetation diversity (Choudhury et al., 2022), which contradicts the results of this study, as denitrification is likely taking place in the natural tidal wetland (the most diverse wetland).

Conclusions

Biomass and vegetation diversity

Overall, peak biomass per area showed no clear trend with respect to either position in a wetland and across wetlands. Neither of the natural tidal sites showed the highest biomass per area compared to the urban sites, even though the natural wetland has a higher N input and has been established longer than the urban wetlands. This implies the factors that determines biomass in freshwater wetlands may be more complex than just the amount of N inputs from inlet sources. Competition from other species with different growth habits, and nutrient inputs from upland runoff sources may also affect total biomass in freshwater wetlands.

Generally, sites with a higher number of *Typha* stems have stems that are smaller in diameter. Peak height is more variable with diameter, depending on the site. This indicates that competition for resources with other individuals may be an important factor in determining *Typha* growth habits: if *Typha* dominates an environment, it can either be more numerous or thicker in diameter, but unlikely both, as there is a limited quantity of nutrients and space.

As expected, the longer established natural wetland interior site shows a significantly higher biodiversity than the urban sites, likely because *Typha* is an aggressive species that

propagates through rhizomes and colonizes disturbed areas like urban wetlands while competing with other species for resources. Constructed wetlands may also be initially planted with monospecific stands.

Carbon

Uncertainties in leaf pack experiments are significant enough that differences in C loss rates among wetlands are difficult to determine. However, as carbon is lost from the *Typha* aboveground biomass, it does not change much isotopically, implying that the primary method of initial carbon loss in the leaf litter is one that does not fractionate C. This method is likely related to leaching of DOC (tidal flushing in the tidal sites), rather than microbial decomposition. Leaching preferentially removes more labile forms of OM, which includes C-rich compounds like non-structural sugars, which means that litter that starts out with a higher C:N ratio and more non-structural compounds are likely to lose more C in the initial stages of decomposition.

By the time leaf litter incorporates itself into the upper layer of the soil, the C shows a higher $\delta^{13}C$ signature in the top layer of the cores than the decomposed litter in the urban wetlands. This suggests that either microbial decomposition is occurring after leaching in the urban wetlands, or that additions of ^{13}C from carbonate dust from urban debris are added to the upper layers of the soil.

Nitrogen

N content and isotopic composition of fresh *Typha* varies among the wetlands in a pattern that is consistent with both N loads and N sources to the wetland. The natural tidal wetland has greater

inputs of N, most of which are from isotopically heavy (δ^{15}N of 10 – 20‰) septic inputs. The urban tidal wetland has an intermediate input of N from septic sources and isotopically lighter fertilizer (δ^{15}N of -3 to +3‰) and atmospheric (δ^{15}N of 0‰) sources, and the urban tidal wetland has a low input of isotopically light atmospheric and fertilizer N. These differences in N loads and sources are shown in the initial composition of fresh *Typha*.

There are few significant changes in N content or isotopic composition as the *Typha* litter decomposes, suggesting that N is not lost in the initial stages of decomposition. By the time the litter incorporates themselves into the upper layer of soil, N loading and source differences are much more apparent. In addition, cores in the tidal wetlands are more enriched than the decomposed vegetation in δ^{15}N, suggesting that an N fractionating process like denitrification is occurring in the litter in both tidal wetlands after initial stages of decomposition (8-9 weeks).

Chapter 4: C and N Soil Core Records in Natural and Restored Freshwater Wetlands

Results

Bulk density, organic matter, and carbon content of marsh cores

The amount of carbon stored in wetland soils is a function of soil carbon density, which can be determined from measurements of bulk density and carbon content at various depths within the soil. To calculate carbon density in each depth horizon, soil bulk density and weight percent carbon are required to determine the mass of carbon in soil. Organic matter fraction and its changes with depth in cores identify the fraction of organic or inorganic sediment preserved at each depth, representing what material is preserved at different times. Relationships among bulk density, organic matter content, and weight percent carbon within marsh cores can help determine the effects of sediment inputs, primary production, and decomposition dynamics on carbon storage both within a marsh (over time) and as a comparison between constructed and natural freshwater wetlands.

Differences in bulk density among sites and within cores provide information on marsh sediment composition and its variations in time and space. Data collected for this study indicate that bulk density increases with core depth across all wetland sites. There was little spatial variation in bulk density patterns with depth in the natural tidal wetland; however, spatial variation was higher in both urban wetlands (Fig. 4.1). The inlet site in the urban tidal wetland (near the source of suspended sediment) has higher bulk density (> 0.25 g/cm^3) than the middle and interior sites (< 0.25 g/cm^3) in the upper marsh cores. The interior site in the urban tidal

wetland below 20 cm has a much higher bulk density (> 1.00 g/cm^3) than the inlet site, reflecting the non-organic constructed gravel base. For this reason, these data points were removed due to being outliers. The interior site in the urban nontidal wetland also has a much higher bulk density (> 1.00 g/cm^3) at depth (> 20 cm) than the inlet site.

Do differences in bulk density correlate with fraction organic matter in the sediment core sections across the three wetlands? The two urban constructed wetlands have basal sediments with high bulk density values (> 0.80 g/cm^3) and low fraction OM ($< {\sim}10\%$ OM) characteristic of mineral sediment, as they were constructed within the past 20 years on gravel and other inorganic sediments. The natural tidal wetland has higher OM fractions than the other sites even where bulk density values are similar, as it is a wetland with a high productivity and has been accumulating organic matter longer than the urban wetlands (Fig. 4.1).

Organic matter fraction decreases with an increase in bulk density values. The relationship of OM fraction to bulk density is significantly different among the wetlands. In core sections with the similar bulk densities, the fraction of OM in the natural tidal wetland is significantly higher than in the other wetlands. Relationships between the bulk density and organic matter fraction are shown in Figure 4.1. The urban wetland cores have similar relationships between fraction OM and bulk density, which are different from the natural tidal wetland's cores. All three wetlands have an exponential relationship between fraction OM and bulk density. Exponents are -1.45, -1.27, and -1.34, for natural wetland, urban tidal, and urban nontidal, respectively. The relationship for the natural tidal wetland is closer to linear ($R^2 = 0.74$), suggesting that the increase in bulk density could be primarily due to organic matter compaction with depth:

$$Frac.OM = -0.2941(BD) + 0.3181$$
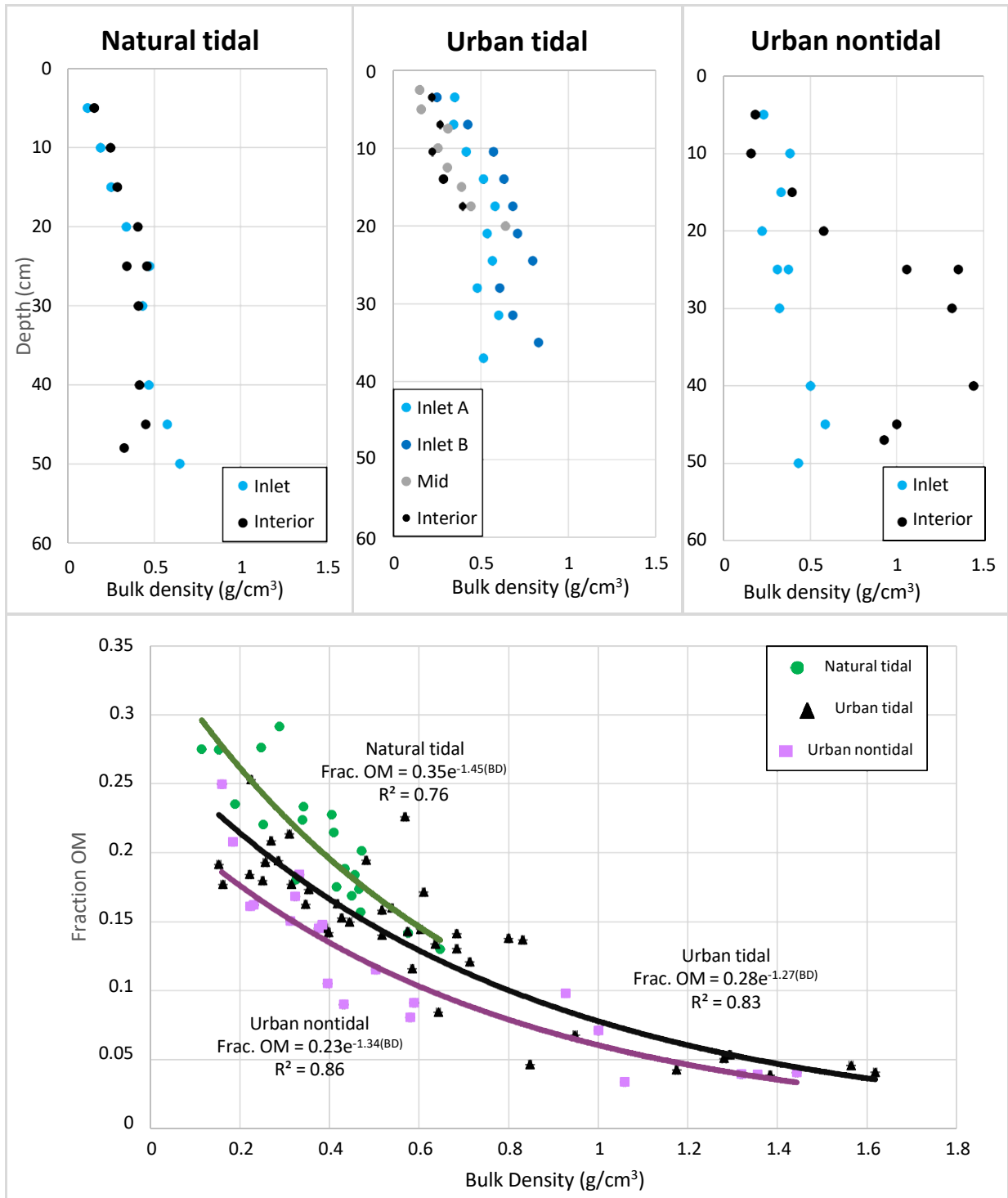
Figure 4.1. Bulk density with core depth and % OM vs. bulk density. (Upper) Core bulk density from 0-50 cm depth at each wetland site. (Lower) Percent organic matter vs. bulk density for all core samples.

The relationship between OM content and weight % C provides information on the value of OM content as a predictor of soil carbon content. Across all sites, weight percent carbon increases linearly with weight % OM with the relationship:

$$\% \, C \; = \; 0.40(\% \, OM) \, - 0.45$$

with an R^2 value of 0.77 (Fig. 4.2).

The carbon content and carbon density at depth intervals in the marsh cores provide data to calculate carbon stocks at various depths in the soil. Carbon content decreases with core interval depth at all sites; however, the natural tidal wetland overall contains more carbon throughout, including at depth, compared to the other sites. In addition, the surface OM and carbon in the natural tidal wetland is much higher than the surface carbon in the constructed wetlands (consistently > 20% OM, > 10% C in natural wetland; Fig. 4.2).

Inlet and interior sites exhibit similar trends in carbon content, except for the urban nontidal site. The nontidal inlet site shows a higher carbon content at rooting depths ($\sim 5 - 8\%$ from 20-40 cm), compared to the carbon content at the rooting depth in the interior site ($\sim 0 - 1.5\%$; Fig. 4.2). As the urban wetlands are both of known ages, the rates of carbon accretion can be calculated knowing the carbon stocks accumulated.
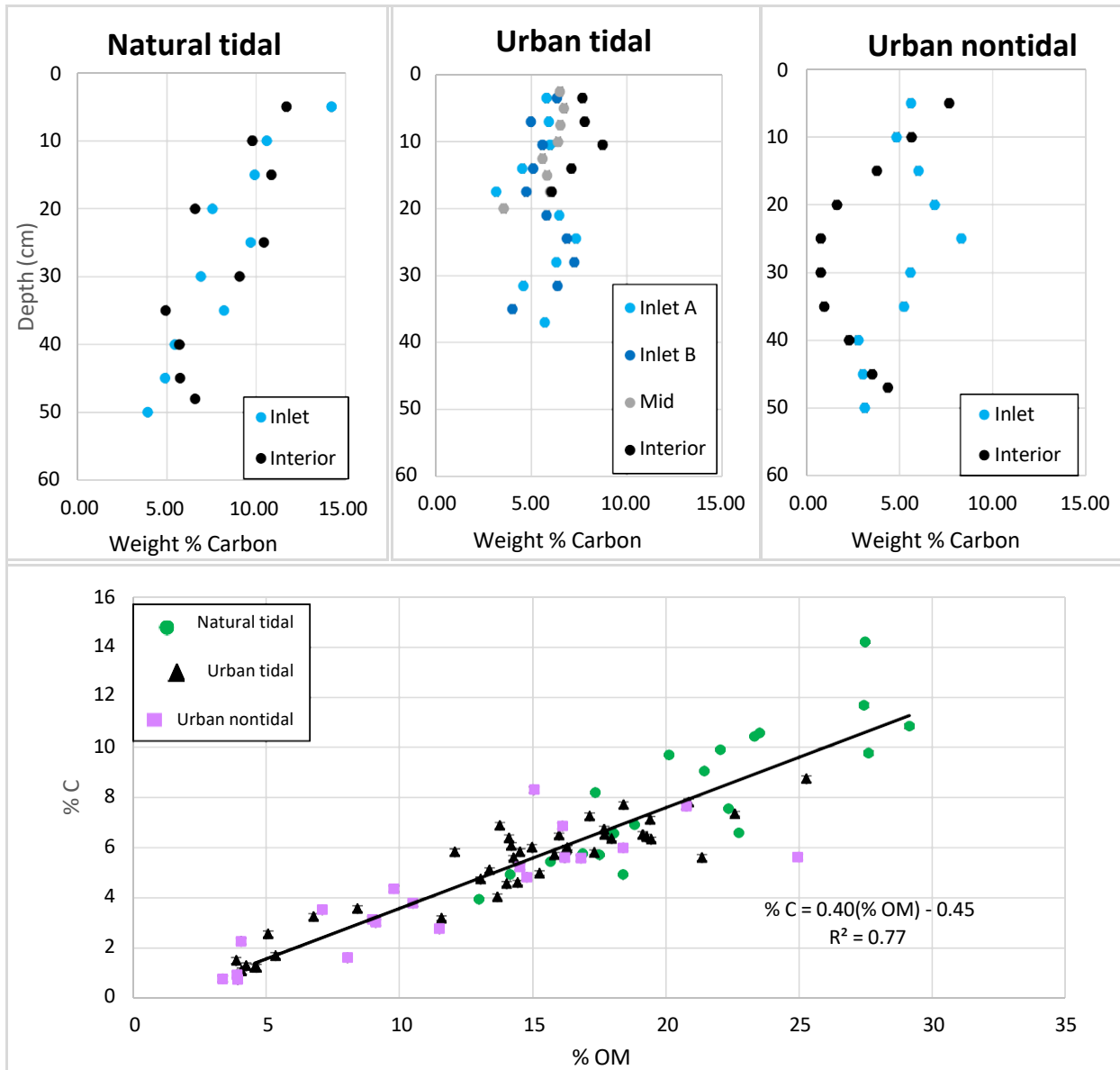
Figure 4.2. Weight % C with core depth and % C vs. % OM. (Upper) Core % C from 0-50 cm depth at each wetland site. (Lower) Percent C as a function of % OM for all core samples.

Carbon stocks in upper 10 cm and upper 30 cm of core

Bulk density and weight percent carbon data were used to determine the carbon stocks for specific depths at each wetland site. The carbon stocks in the upper 30 cm include the carbon that has accumulated over longer timescales, and the carbon stocks in the upper 10 cm include the

carbon that has accumulated more recently. The natural tidal wetland has little spatial variation in carbon stocks in the upper 30 cm and has consistently high carbon stocks compared to the constructed urban wetlands, likely due to the high organic matter content of the sediment from high primary productivity and long wetland age. This is also evident in the carbon stocks in the top 50 cm of the natural tidal wetland. The inlet has 14 g C per m^2 and the interior has 13 g C per m^2 in the upper 50 cm of soil depth, showing no large spatial variations in C storage across the natural wetland. The two urban wetlands have higher spatial variability in carbon stocks than the natural wetland. Most constructed urban sites examined have similar or lower carbon stocks than the natural tidal wetland; however, the high carbon stocks in the upper 30 cm of the nontidal wetland (11.25 kg C per m^2) may represent the accumulation and retention of organic-rich mineral sediments (silt) as the site transitioned from a pond to a marsh. Recent (upper 10 cm) marsh accretion at the nontidal interior site indicates lower carbon stocks than the inlet site (0.90 kg C per m^2 and 1.67 kg C per m^2 respectively), perhaps due to recent dumping of vegetation clippings near the inlet site (Fig. 4.3).

Soil carbon stocks that accumulated in a known time interval can be used to determine the average rate of carbon accretion. The time period for wetland carbon accumulation in the urban tidal wetland is known. The urban tidal wetland contains only around 30 cm of organic material. The wetland was constructed in 2010 by dredging sediment from the banks of the Anacostia, and then laying a layer of gravel on top of the cleared area. The amount of carbon accreted in the 27.5 cm-deep middle site core is 0.58 kg C per m^2. Therefore, the rate of carbon accretion for the middle of the wetland since construction in 2010 are 576 g C per m^2 per year.
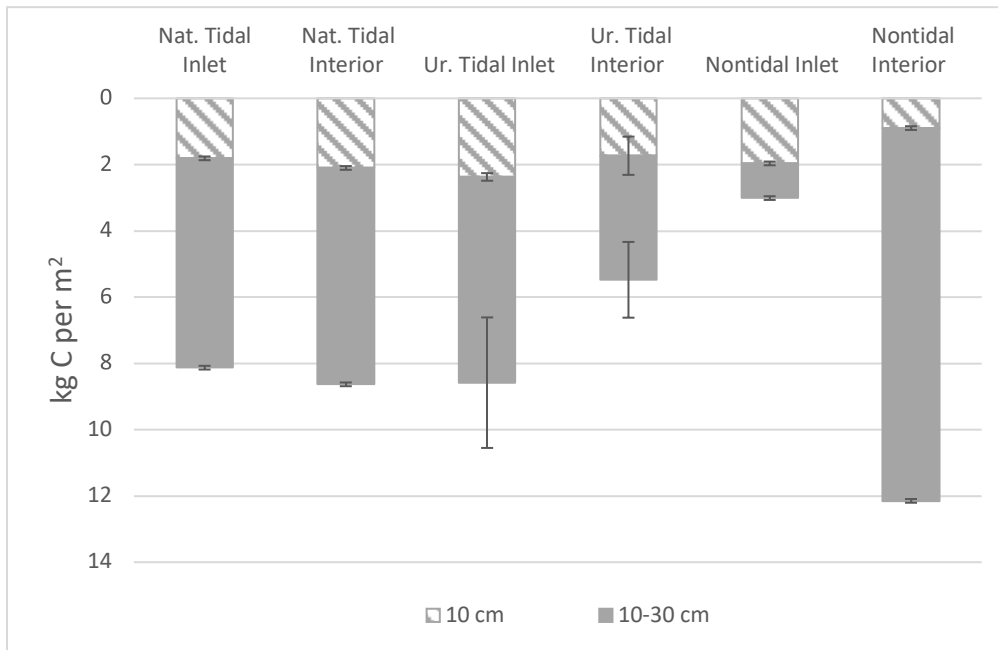
Figure 4.3. Carbon stocks. Carbon stocks at each wetland site for the upper 10 cm and from 10 – 30 cm depth.

Carbon isotope variations in marsh cores

Carbon isotopic compositions for the core data are shown in Figure 4.4. These data indicate that carbon isotopic composition is similar throughout all natural tidal core samples and the urban core samples with carbon contents of 4% C and higher. The δ^{13}C of these cores range between -24‰ to -29‰. The mixing curve in Figure 4.4 shows that urban core samples with low C concentrations display large variations in the relationship between δ^{13}C and 1/concentration of C, while natural cores and high-C urban core samples show a tight range of δ^{13}C values.

Carbon isotopes are consistent between the fresh *Typha*, the decomposed *Typha*, and the high-carbon core samples. The δ^{13}C of the *Typha* vegetation ranges from -29 to -30‰. Carbon isotopic composition of the high-carbon samples is similar to the δ^{13}C of the *Typha* and does not change significantly with depth in the two tidal wetlands (Fig. 4.4). In the two tidal wetlands, there is little fractionation of C with depth, just as there is little fractionation of C in

decomposing vegetation over time, suggesting that non-fractionating processes like tidal leaching or flushing are important in removing carbon in both the leaf litter and the belowground organic matter in the cores. The nontidal wetland interior, however, is enriched in ^{13}C at the rooting depth (-24.65‰ to -24.45‰ from 20 – 40 cm depth) compared to the inlet site.
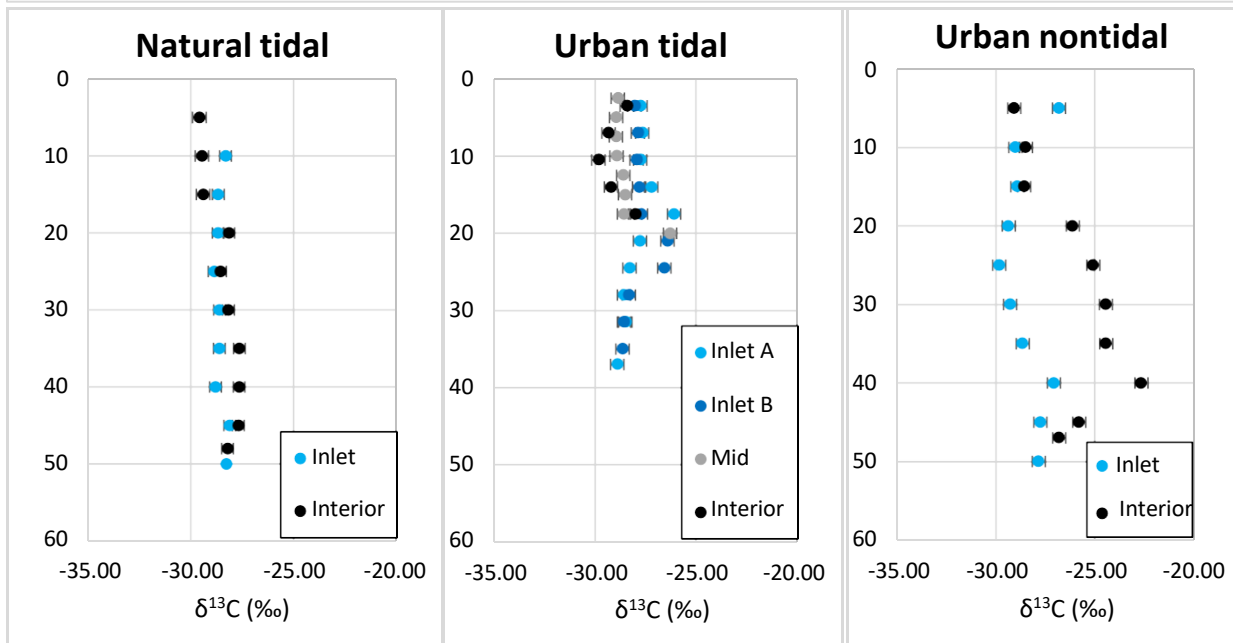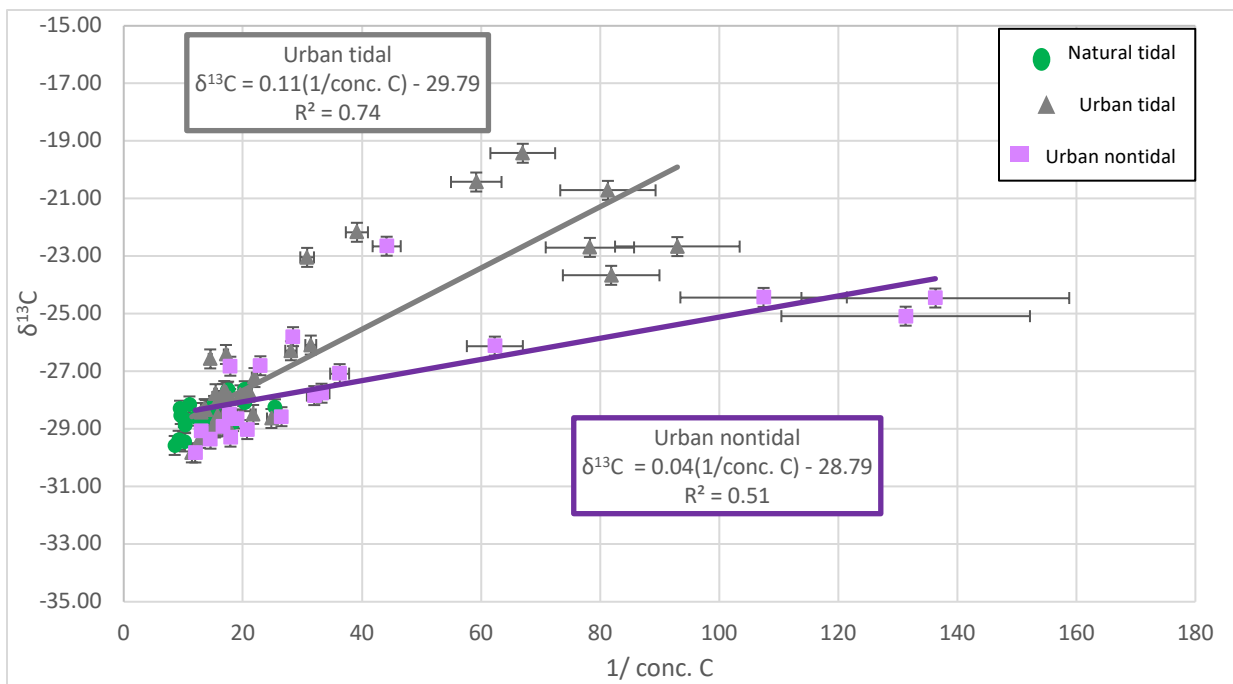
Figure 4.4 δ¹³C mixing curve and δ¹³C with core depth (Upper) Mixing curve showing $\delta^{13}C$ with 1/concentration C of core samples from 0-50 cm depths across all wetland sites. (Lower) $\delta^{13}C$ with depth in the sediment cores across the wetland sites.

Most core samples from the three wetlands have similar $\delta^{13}C$ to each other, but their C:N ratios are different (Fig. 4.5). Except for outliers in the lower, inorganic parts of the constructed urban cores, the relationship of all the cores $\delta^{13}C$ to C:N ratios is similar. They fall within and between the ranges outlined by Khan et al. (2015) for C3 plant-dominant marshes and freshwater upland wetlands (Fig. 4.5).

The C:N ratios with depth range from ~10 – 20 for most sites, with three exceptions. The first two exceptions are from the urban tidal wetland. One of the inlet cores has a C:N ratio of 21.99 at 21 cm depth and the interior core has a ratio of 25.35 at 17.5 cm depth. The second exception is the entire interior core in the nontidal wetland below 25 cm (C:N ratios of 20.89 to 54.22; Fig. 4.5).
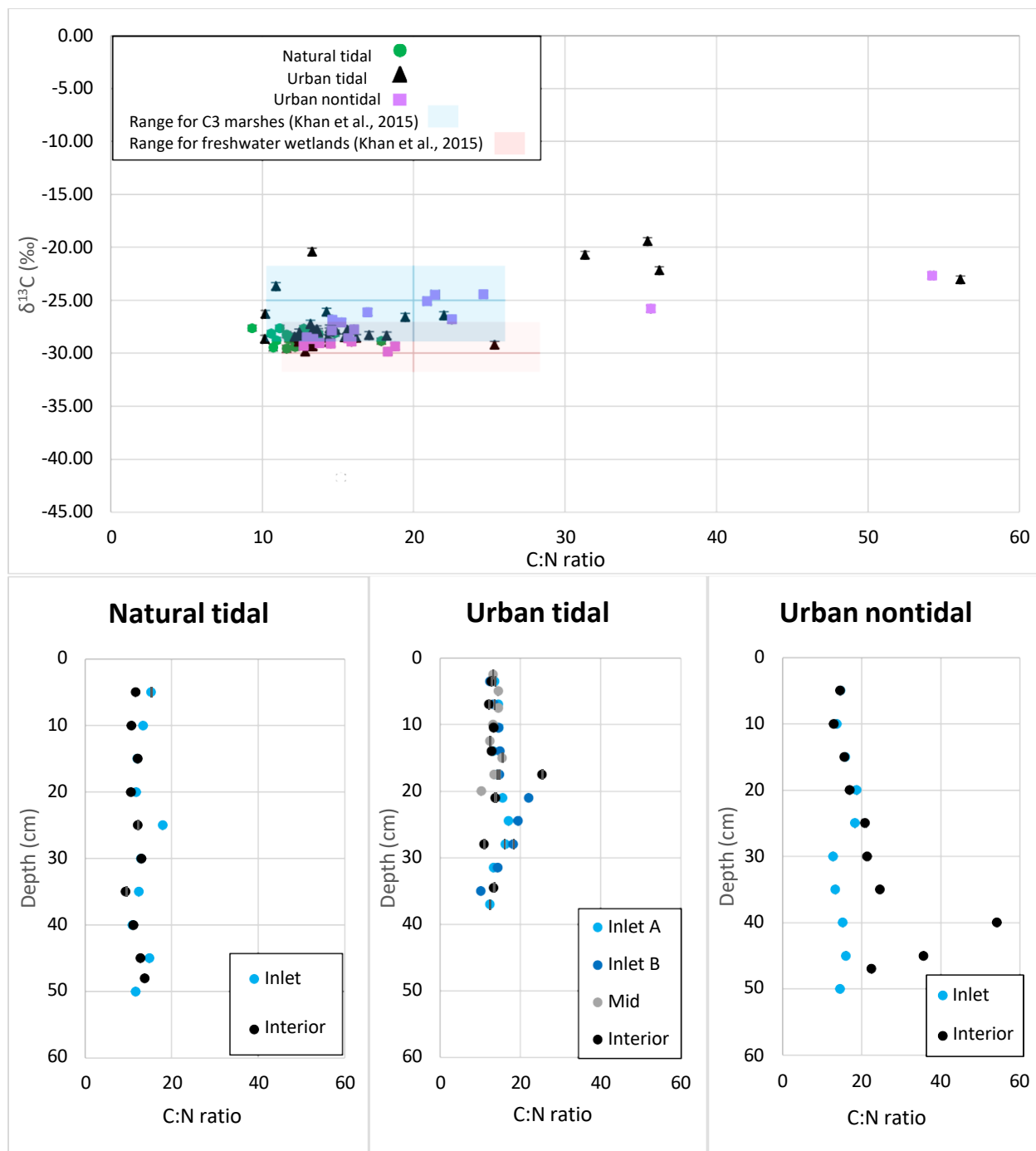
Figure 4.5. C:N ratios with core depth and δ¹³C vs. C:N ratio. (Upper) $\delta^{13}C$ vs. C:N ratios of core samples across wetland sites. (Lower) C:N ratio with depth in the sediment cores across the wetland sites.

Nitrogen distribution in marsh cores

Nitrogen content and bulk density measurements were used to determine nitrogen storage in the upper 10 cm and 30 cm of the marsh cores. Nitrogen content of the vegetation was highest in the natural tidal wetland (Chapter 3), and nitrogen stocks are consistently higher in the natural tidal wetland (0.58 to 0.73 kg N per m^2) and lower and more variable in both constructed urban wetlands. The interior sites in the two urban wetlands contain less N (0.34 and 0.21 kg N per m^2 in the urban tidal and nontidal wetlands respectively) in the top 30 cm of soil than the inlet sites of their respective wetlands (0.54 and 0.77 kg N per m^2). Overall, the N stocks in the top 10 cm of sediment is less variable across all wetland sites, and most of the variation in N storage in the cores comes from the N stored from 10-30 cm of depth (Fig. 4.6).
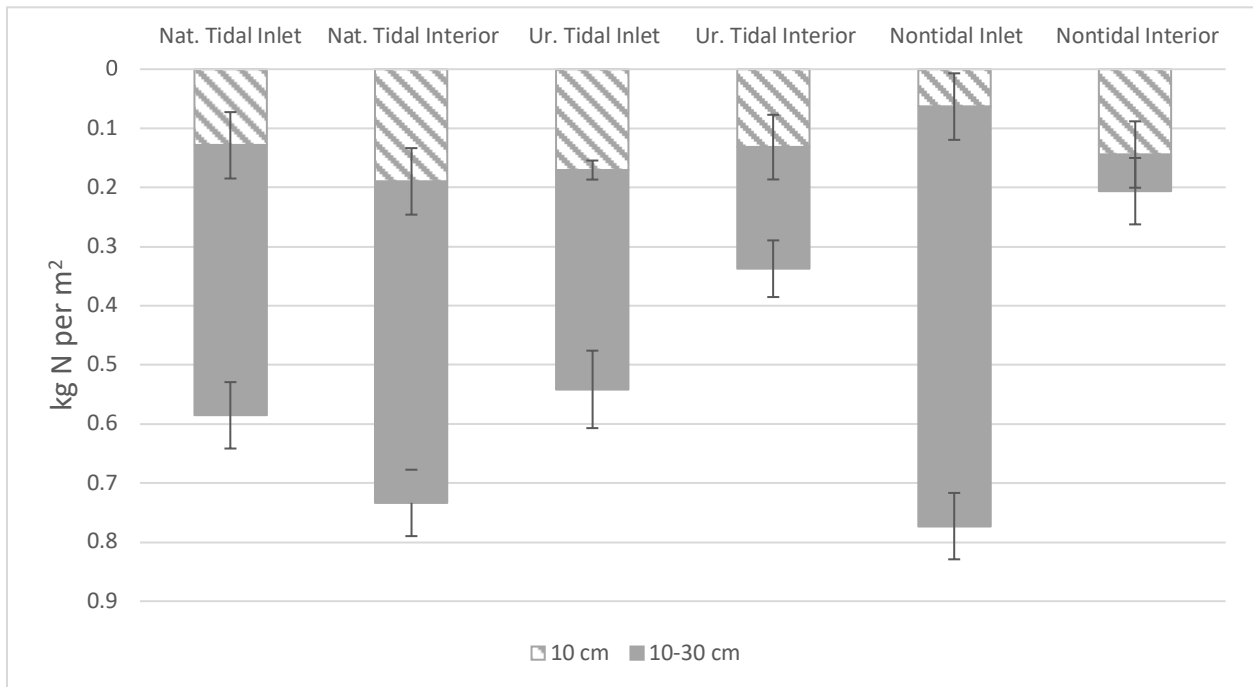
Figure 4.6. Nitrogen stocks. Nitrogen stocks at each wetland site for the upper 10 cm and from 10 – 30 cm depth.

Nitrogen content decreases with core increment depth in both tidal wetlands. In the top 15 cm of the urban tidal wetland, the interior site shows higher percent N (0.56% to 0.66%), but it decreases at depth.

The urban tidal core from 20 cm onward in depth has an extremely low % N (~ 0%) and therefore did not allow for a precise measurement of % N. The urban nontidal core is the only wetland that shows a large discrepancy between the interior and inlet with depth. The interior quickly loses nitrogen after 20 cm in depth, and the inlet gains nitrogen. At the base of the core, the nitrogen content of both inlet and interior converges again at ~ 0.20% N (Fig. 4.7).
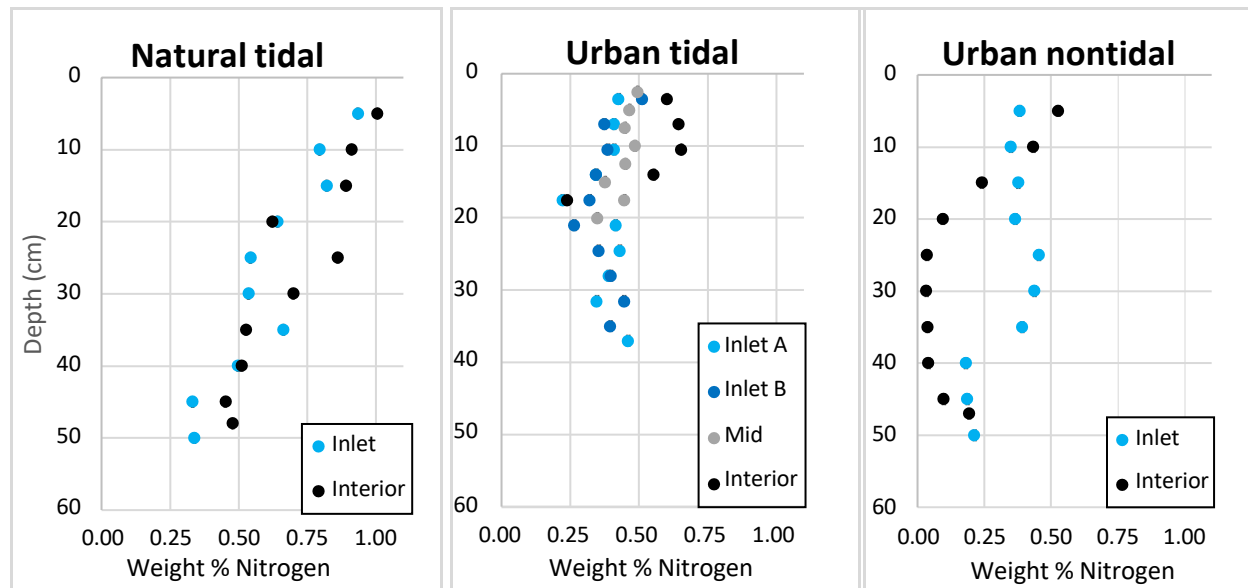
Figure 4.7. Weight % N with core depth. Core % N from 0-50 cm depth at each wetland site.

Nitrogen isotopic composition of cores

Comparison among the sites indicates that N content and isotopic composition vary significantly among the wetlands. Overall, the nontidal wetland has the lowest core N content and lowest δ^{15}N, the natural tidal wetland has the highest % N and δ^{15}N, and the urban tidal wetland has values in between the other two. These variations among the sites are also reflected in variations in the decomposed and

fresh Typha values. The % N values of the vegetation are higher than core values. In the nontidal wetland, the decomposed vegetation has similar $\delta^{15}N$ values to the upper part of the core (~ 4‰). In both tidal wetlands, the decomposed vegetation has lower $\delta^{15}N$ values (~ 7 – 10‰ in natural tidal, ~ 3 – 5‰ in urban tidal) than the isotopic values of the upper cores (~ 11 – 12‰ in natural tidal, ~ 6 – 7‰ in urban tidal; Fig. 3.2 and Fig. 4.8).

Nitrogen isotopic values and their changes with depth are different for the three wetlands. Nitrogen isotopic values are ~ 11 – 13‰ in the natural tidal wetland and values decrease with depth in each core. The interior site is more enriched in ^{15}N than the inlet site. In the urban tidal wetland, nitrogen isotopic composition does not shift significantly with depth other than at the interior site, which shows an enrichment ^{15}N in the 7 – 14 cm depth range ($\delta^{15}N$ of 6.93 – 7.22‰ from 7 –14 cm depth; 5.27 – 7.40‰ at other depths). Nitrogen isotopic values are lowest in the nontidal wetland. Comparison of the inlet and interior sites indicate a shift towards the atmospheric mean ($\delta^{15}N$ of 0‰) near the rooting depth (< 20 cm) in the interior urban nontidal site (Fig. 4.8).
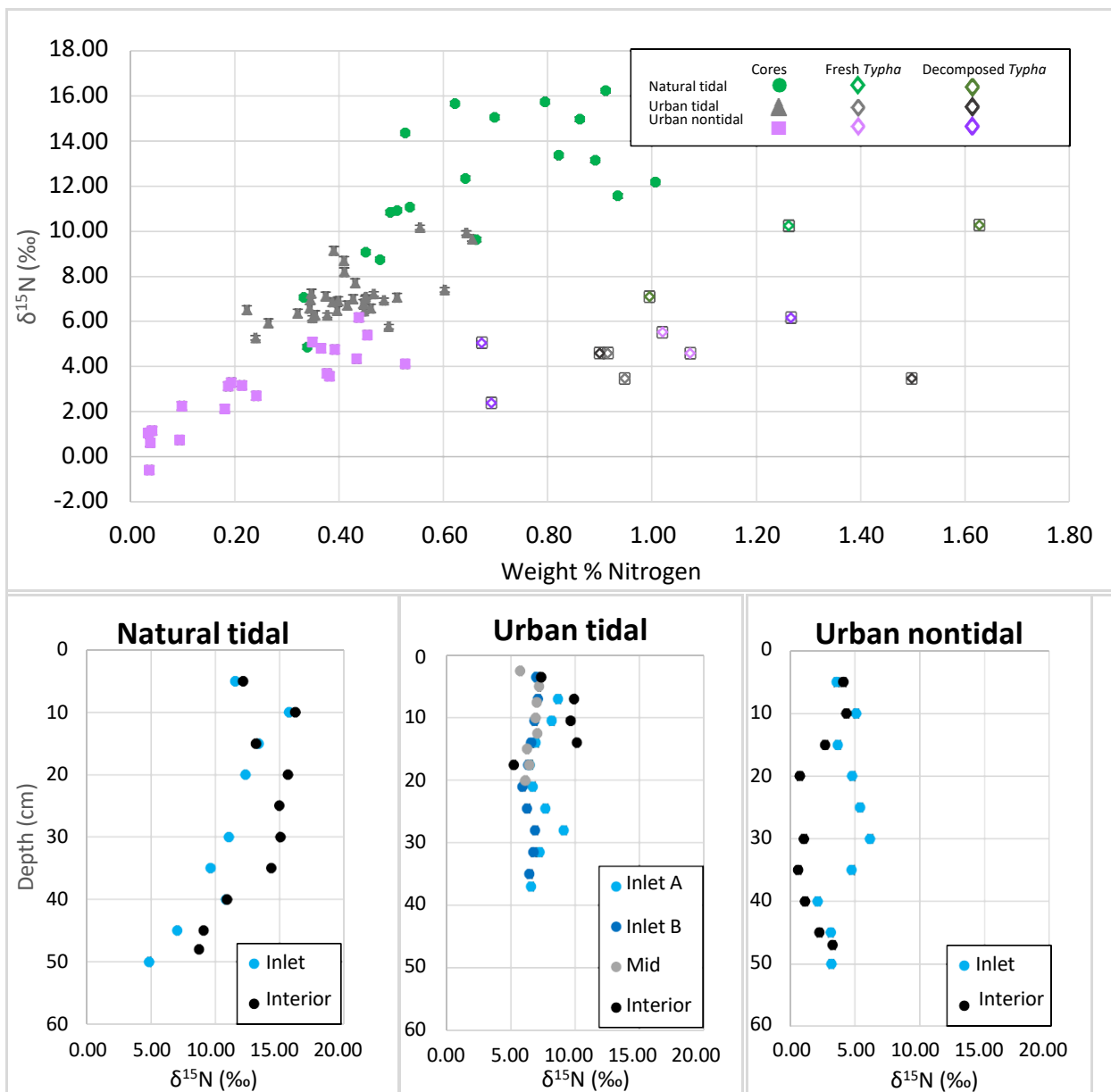
Figure 4.8. δ^{15}N with core depth and δ^{15}N vs. % N. (Upper) δ^{15}N vs % N of core samples from 0 – 50 cm depths across all wetland sites. (Lower) δ^{15}N with depth in the sediment cores across the wetland sites.

Discussion

Bulk density, organic matter, and carbon content of marsh cores

Other temperate freshwater wetlands reported in the literature have a wide range of OM

compositions. For example, an Ohio depressional marsh without external sediment sources was

98 – 99% OM (Bernal and Mitsch, 2012); a natural Indiana marsh had an OM content of ~ 13%

(Ferrara et al., 2020); and tidal freshwater Virginia marshes have 35 – 38% OM (Morrissey et

al., 2014). The wetlands in this study have OM values that range from < 5% (near the base of

the urban wetland cores) to around 30% (the surface of the natural wetland), placing them as

relatively OM-poor compared to other freshwater tidal wetlands. However, the study wetlands

have a range of core total C similar to the range of other temperate freshwater wetlands: for

example, 2 – 12% C in cypress wetlands in Florida (Powell et al., 2019), 13% surface C in

forested tidal wetlands on the Apalachicola river in Florida (Anderson and Lockaby, 2011), and

8 – 14% in a Georgia tidal marsh (Craft, 2007). The wetlands in this study have a range of % C

from < 2% (near base of the urban wetland cores) to ~ 14% (surface of natural wetland). This

puts them in the same range of soil % C as other freshwater wetlands.

      The relationship between % C and % OM in the cores is linear and positive (Fig. 4.2).

The relationship between the average % C and % OM of the fresh and partially decomposed

Typha aboveground vegetation follows the trend of the cores (average OM percentage of

vegetation is 87%; average % C is 32.9%; ratio is 2.64), suggesting that the processes that start

removing OM from vegetation at the surface are similar to the processes that remove OM from

soil at depth.

      For these three wetlands, % OM can predict % C according to the linear relationship

$$\% \, C \; = \; 0.40(\% \, OM) \; - 0.45$$

$$R2 \; = \; 0.77,$$

but this relationship may not hold in other freshwater wetlands as they tend to have a higher % C with a given % OM.

The highest % OM among the study wetlands is found at the surface of the natural tidal wetland (27.47%; Fig. 4.1). As the surface is where both organic and inorganic carbon accrete, the higher OM percentage in the surface of the natural wetland could be due to either a higher primary productivity compared to the constructed wetlands, less influx of inorganic sediment to the marsh platform compared to the constructed wetlands, or both. As decomposition rates of surface vegetation are similar and primary production shows no clear patterns across all wetlands, the lower surface carbon and OM in the constructed wetlands is probably due to higher influxes of inorganic sediment instead of faster decomposition or primary productivity.

Carbon content generally decreases with depth across all wetland sites, with a discrepancy between the inlet and interior sites from 20 – 40 cm depth in the nontidal wetland (Fig. 4.2). This higher % C at the inlet site (5.24 – 8.31% C) compared to the interior (0.93 – 1.60% C) could be due to the flux of dense silty pond sediment from the inlet to the interior of the marsh during the early period of marsh formation, or to dumping of leaf litter and grass clippings near the interior site.

Carbon stocks in upper 10 cm and upper 30 cm of cores

The natural tidal wetland exhibits overall higher % C and % OM amounts within the soil cores; the natural tidal wetland also has consistently higher carbon stocks in the upper 30 cm compared to the urban wetlands (Fig. 4.3). The urban wetlands have spatially variable C

stocks due to changes in C content with depth and location in the wetland. Because the natural wetland is organic through 50 cm, and the urban wetlands reach mineral horizons between 20 and 40 cm depth due to shorter periods of organic matter accretion, wetland age is likely the main factor contributing to differences in carbon stocks: older wetlands store more carbon because they have thicker organic horizons that have accreted over time.

Carbon stocks vary with location in both urban constructed wetlands, which might reflect sediment dynamics during recent wetland formation. The reason the interior nontidal site shows higher C stocks from $10 - 30$ cm depth (11.25 kg C per m^2) may be due to dense silty sediment transported from the inlet to the interior site, which was a pond during early stages of marsh formation.

Freshwater wetlands on the coastal plain of the Eastern U.S range greatly in C accretion rates: from $22 - 75$ g C per m^2 per year in tidal forested southern wetlands (Craft, 2012), from 50 to over 400 g C per m^2 per year in tidal freshwater marshes (Drexler et al., 2013). In this study, wetland on the Anacostia freshwater tidal wetland has a much higher C accretion rate (576 g C per m^2 per year) than other similar wetlands. This could be because this study's urban tidal wetland is extremely new (constructed in 2010) and is in the early stages of carbon accretion. Wetlands may exhibit a much higher apparent rate of recent carbon accumulation compared to long-term carbon accumulation, because of leaching and decomposition over time (Bao et al., 2011). The Anacostia River urban tidal wetland exhibits an even higher rate of C accumulation than even the fastest C-accumulating wetlands in other studies. The fastest C-accumulating wetlands tend to be the naturally tidal ones, likely because tidal wetlands are flooded daily and dry out less often, leaving less opportunity for oxidation of organic matter (Drexler et al., 2013). It follows then that the urban tidal wetland in this study should have a high C accretion rate. The reason the rate is so much higher than the rates in the literature could also be because

it is accumulating inorganic carbonate from dust from the cement plant nearby.

Carbon isotopes

The relationship of $\delta^{13}C$ to 1/concentration C for core samples with C contents above ~ 4% indicates that losses of carbon from high-carbon core samples (i.e., organic parts of cores) occur without major shifts in isotopic composition, as in the decomposing vegetation. This implies that the dominant method of organic carbon loss in the vegetation and cores is one that does not fractionate C, such as tidal flushing or leaching (Álvarez and Bécares, 2006; Ibanez et al., 1999).

The mixing curve in Figure 4.4 shows that the natural wetland cores and the organic parts of urban wetland cores have similar C content and isotopic compositions, while the inorganic parts of the urban wetland cores have a wider range of $\delta^{13}C$ values. The urban tidal wetland has a different low-C end member $\delta^{13}C$ composition than the nontidal wetland (Fig. 4.4), likely because the sources of the C in the inorganic core sections are different.

Differences in $\delta^{13}C$ values between the inorganic portions of the urban tidal wetland and the urban nontidal wetland cores are also visible in the data showing $\delta^{13}C$ with depth. The urban tidal interior and middle site core samples have very low % C values (~ 1.00%) and enriched $\delta^{13}C$ values (-19.43‰ to -23.04‰, Fig. 4.4). These samples, also visible in the mixing curve, are likely due to carbonate cement dust from a nearby cement plant, since both lithogenic and carbonates in soil tend to have more enriched $\delta^{13}C$ compositions (-0 and -25‰ respectively; Washbourne et al., 2012). These outliers will be excluded from the discussion of the remaining core samples.

The urban nontidal wetland shows a different relationship between $\delta^{13}C$ and

1/concentration C (Fig. 4.4). The nontidal wetland displays an enrichment of ^{13}C near the rooting depth in the interior of the nontidal wetland (δ^{13}C of -26.12‰ to -22.65‰; Fig.4.4) that is slightly lower than the δ^{13}C values previously discussed in the urban tidal wetland. The source of C to these samples in the nontidal wetland may be an influx of pond sediment enriched in ^{13}C from inlet to interior during an early period of marsh formation. Organic sediment in a Spanish, groundwater-fed, recently created shallow freshwater pond has a higher δ^{13}C than the sediment in the study wetlands (-13‰ to -26‰ for pond sediments compared to ~ -25‰ to -30‰ for the study wetlands; Rodrigo et al., 2016). While the pond studied by Rodrigo et al. (2016) is in Spain and not on a golf course, it is freshwater, groundwater-fed with an outlet, and recently constructed at the time of the study, roughly approximating the conditions of the pond the UMD golf course before formation of the nontidal wetland. Therefore, the section of the nontidal interior core that has a higher δ^{13}C may be due to pond sediment travelling to the interior as the nontidal wetland formed from the inlet inwards.

The enrichment in δ^{13}C in the interior site of the nontidal wetland may also be due to contamination from grass clippings and leaves. The nontidal wetland is on a golf course, so this isotopic shift could be from a pile of clippings and leaves from course management dumped near the interior site. As the fairway and rough consist of bermudagrass (a C4 grass) and tall fescue (a C3 grass), there may be some C4 plant material deposited next to the interior site. C4 plants are more enriched in ^{13}C than C3 plants (C4 plants have tissues with δ^{13}C values from -12 to -14‰, C3 from -26 to -29‰; Fernandez et al., 2003), so the enrichment in the isotopic composition of the interior site may be due to the dumping of grass material nearby.

The core data also indicate that most of the sediment in the study wetlands falls within a reasonable range of C:N and δ^{13}C values for C$_3$ marshes to freshwater uplands and the area in between (Khan et al., 2015). The exceptions are a few lower (> 20-30 cm depth) core samples in

the interior sites of the urban wetlands, which have high C:N ratios (> 20) and are likely

contaminated either with carbonate dust from a factory or urban area or gravel residue from the

constructed bases and have been excluded from much of the discussion (urban tidal) or contain

dense silty pond sediments from early formation (urban nontidal). The range of core isotopic

values makes sense as the three wetlands are dominated by *Typha* and other C3 plants

(composites, *Carex* sp. sedges, *Leersia* sp. cutgrass, etc.).

  C:N ratios at each site are similar, except for an inlet and an interior core from the urban

tidal at the start of the rooting depth (~ 20 cm) and the interior core in the nontidal wetland

below 30 cm (Fig. 4.5). The high C:N ratios (21.99 in inlet, 25.35 in interior) near 20 cm depth

in the constructed tidal wetland are likely not due to carbon additions, as the % N decreases at

this depth; they are likely not due to denitrification, as N isotopes do not change significantly at

this depth. The high C:N ratios may be due to an uptake of N at the rooting depth of the plants.

  The higher C:N ratio in the interior of the nontidal wetland (21.41 – 54.21) near the base

of the core maybe due to the presence of dense, N-poor pond sediment from the construction of

the wetland. The wetland accreted from the inlet inwards, so organic muck, algae, and biomass

would have travelled to the interior from the inlet during the early stages of wetland formation.

This would add carbon to the pond sediments near the interior.

Nitrogen distribution in marsh cores

The lack of variation in N storage near the surface of the cores among the three sites could be

due to the similarities in N content of the vegetation before decomposition or leaching, which

could ultimately set by the sources of N to the wetlands. While the N content of the fresh

vegetation is statistically significantly different between sites, these differences are not large.

The % N of the fresh *Typha* aboveground biomass differs less than 0.5% between most sites, so

the lack of variation near the top of cores is reasonable (Fig. 4.7). The large variations beneath 10 cm of depth could be due to N sources in the past or the dynamics of vegetation uptake of N in different environments.

The natural tidal wetland is not limited in N inputs, so any spatial variation in soil N due to plant uptake may not be very significant. However, both urban wetlands are poorer in N inputs (especially the nontidal wetland, as the transport of N from the inlet to the interior is slow). Plants near the wetland inlet receive nitrogen from the slow (10-30 m/day) inflowing shallow groundwater, but after these inlet plants take up incoming N there may be little left in water in the interior of the wetlands, as the shallow groundwater takes several weeks to traverse to the interior of the wetland (Prestegaard, pers. comm.). Plants on the interior must take up N in the soil instead of from the water. This model (as in Cecchetti et al., 2020) explains that below the rooting depth in the interior of N-limited wetlands, there is less soil N storage than at the inlet. This idea is also suggested from the relationship between N isotopic composition and N content, which shows a linear mixing between low N values with $\delta^{15}N$ values near 0‰ and higher values of % N and $\delta^{15}N$.

The model from Cecchetti et al. (2020) could explain the differences in N content below the rooting depth in the N transport-limited nontidal wetland. The interior site loses almost all N below 20 cm of depth, while the inlet site maintains its % N or even shows a slight increase below the rooting depth (Fig. 4.7). This difference in % N could be due to plant uptake of N at the inlet, leaving the interior N-poor below the rooting depth. Because N is delivered to the nontidal wetland primarily through slow shallow groundwater flow (Prestegaard, pers. comm.), the residence time of N near the inlet is high, giving plants time to take up the N before it reaches the interior. The long residence time of shallow groundwater in the nontidal wetland affects spatial differences in N in cores and vegetation.

While the uptake model described above affects spatial distributions of N, it does not affect spatial C distributions directly, as C content and isotopes are more influenced by inundation frequency, primary production, and sediment dynamics. However, sediment dynamics may also influence N distributions, especially in the urban nontidal wetland, which was a pond at the interior when initially constructed.

Constructed golf course ponds may have sediment with very low % N compared to wetland sediment (0 – 0.01% N and 0.1 – 0.13% N, respectively; Aelion and Warttinger, 2009), which is consistent with the low N content and stocks (0.06 kg N per m^2) below 10 cm depth in the interior site. The low N stocks therefore could be due to either plant uptake or pond sediment during construction.

Nitrogen isotopic composition of cores

Overall, the relationship between core $\delta^{15}N$ and % N reflects N sources to the three wetlands in both magnitude and type of N loading. The wetlands with larger N inputs have higher % N throughout their cores, and the wetlands with more N from septic sources have more enriched $\delta^{15}N$ signals (10 – 20‰; Valiela et al., 2000). However, the decomposed *Typha* in both the tidal wetlands has a range of $\delta^{15}N$ that is overall more depleted in ^{15}N (3.47 – 10.27‰) than in the upper cores (5.76 – 12.18‰; Fig. 3.2). Vegetation that becomes heavier in N isotopic composition in the process of turning into SOM indicates that there is a process that is fractionating N between the decomposing vegetation and the soil. This process is likely to be denitrification, which is likely a process that occurs in the channels of the natural tidal wetland (Seldomridge and Prestegaard, 2012).

As N content and isotopic signatures vary with depth through the cores, examining

changes to N sources over time may help to decipher these variations. While atmospheric

deposition, fertilizer inputs, and wastewater are common sources of N to wetlands, they have

changed over time, and these changes may be visible in the N core records of the wetlands, or

they may be masked by effects of decomposition or plant uptake. Can the cores from these

wetlands be examined to determine whether N usage or N sources are more important in

recording N over time?

The atmospheric N deposition in the Maryland coastal plain area has decreased

significantly over time. Since 1989, N deposition has decreased from ~ 6.0 kg N per ha to < 4.0

kg N per ha (NTN Sites MD13 and MD99). It follows that the atmospheric contributions of N to

the wetlands will decrease compared to the wastewater and fertilizer contributions of N.

However, the contributions of wastewater and fertilizer N to both river-fed tidal wetlands have

also been decreasing over time (Mason, 2021; Moyer and Blomquist, 2020), meaning that

overall, all types of N loads to all wetlands are decreasing. Isotopically, the natural wetland first

gets more enriched in ^{15}N with depth, then slowly gets less enriched, showing that over the time

of accretion of the core, the nitrogen gets heavier, then, recently, lighter (Fig. 4.8). As a heavier

δ^{15}N (10 – 20‰) is associated with septic sources and a signature closer to 0‰ is associated

with the atmosphere (Valiela et al., 2000), the natural tidal wetland over time has increased in

septic N inputs, and then recently decreased as efforts to control pollution have improved

(Mason, 2021; Moyer and Blomquist, 2020), while atmospheric inputs have decreased over time,

but not enough to override the changing septic source signal.

In the urban nontidal wetland, δ^{15}N only shows spatial variations with depth below the

rooting zone (~ 20 cm depth; Fig. 4.8). These variations may be due to pond sediment in the

interior from the early stages of wetland formation; these variations may also be due to the

depletion of N in the interior because of uptake of fertilizer runoff N by the plants at the inlet

(according to the model of plant uptake by Cecchetti et al., 2020). However, the $\delta^{15}N$ signatures in the interior site suggest that plant uptake may be overriding the signals from historical sediment dynamics. Golf course pond sediment has $\delta^{15}N$ values of 4.6 – 9.8‰ (Kumar et al., 2016). The $\delta^{15}N$ of the interior site below the rooting depth approaches 0‰, which is the atmospheric $\delta^{15}N$ value. The N isotopic composition suggests that the vegetation may be taking up fertilizer N before shallow, N-rich groundwater reaches the interior, as in Cecchetti et al, (2020). While the interior site contains dense, N-poor pond sediment below 20 cm, N isotopes suggest that plant N usage dominates core signatures in the N-limited urban nontidal wetland.

Conclusions

Bulk density, organic matter, and carbon content of marsh cores

The relationships between bulk density and percent organic matter in the cores show that, as expected, bulk density decreases as cores have more OM. However, as the natural tidal wetland is primarily organic past 50 cm of depth, it compacts evenly, unlike the urban cores, which reach mineral horizons before 50 cm depth. Therefore, the relationship between bulk density and % OM is closer to being linear for the natural tidal wetland, showing how it is more evenly compacted throughout 50 cm of depth.

Overall, the three wetlands in this study are relatively poor in OM compared to similar wetlands, yet their soil profiles contain expected amounts of total C. This may be because two of the wetlands are newly created, and therefore have not had time for OM to accumulate and receive inorganic inputs of C through carbonate dust from construction projects. The natural tidal wetland has also been shown to trap significant amounts of inorganic sediment as well, some of which may contain inorganic C.

The relationship between % C and % OM is similar for both the cores and fresh and decomposed *Typha*, suggesting that the processes that remove C and OM are the same for the vegetation and the soil profile. These processes largely do not fractionate C and are therefore not microbial – it is likely that leaching or tidal flushing is an important flux of carbon away from both the leaf litter and the soil.

The highest % C and % OM values are found in the natural tidal wetland, although C removal rates during vegetation decomposition are similar across all wetlands. If decomposition rates do not vary across wetlands, yet wetlands still show differences in C and OM content, this implies that other factors, such as inorganic sediment influxes and wetland age, are more important in deciphering down-core records of C and OM.

Carbon stocks in upper 10 cm and upper 30 cm of cores

Carbon stocks in the upper 10 cm of the marshes are similar among the marshes, but stocks in the upper 30 cm of the cores are significantly higher in the natural tidal wetland largely due to wetland age and a longer period of organic matter accretion. In the urban wetlands, sediment dynamics during the early period of wetland creation are important in determining carbon stock patterns. For example, variability in carbon stocks between the inlet and the interior in the nontidal wetland likely reflect how organic pond sediment travelled from the inlet towards the outlet during the early stages of marsh formation.

The C accumulation rate (g C per m^2 per year) was calculated as 576 g C per m^2 per year for the urban tidal wetland. This is significantly higher than typical C accretion rates in other similar wetlands (Craft, 2012; Drexler et al., 2013). The high C accumulation rate in the middle site of the urban tidal wetland may either be due to the accumulation of inorganic C from

cement dust from a nearby cement plant, or to the young age of the wetland.

Carbon isotopes

C isotopes and C:N ratios are consistent with other similar wetlands (C3 and freshwater; Khan et al., 2015). In the organic portion of all cores (entire natural tidal core, and upper portions of urban cores), C isotopes do not significantly change with depth while C content decreases, implying that C is lost without fractionation. This is consistent with how C is lost in decaying leaf litter: primarily by leaching or tidal flushing.

However, the mixing curve of $\delta^{13}C$ with 1/concentration C shows that there two separate sources of C to the deep, inorganic portion of the urban wetlands' cores. In the urban tidal wetland, this source of C could be carbonate dust from a nearby cement plant. In the urban nontidal wetland, the high $\delta^{13}C$ values in the interior site may be due to either past dumping of C4 grass clippings near the interior site, or to influxes of pond sediment to the interior during marsh formation.

Nitrogen distribution in marsh cores

Percent N near the surface of the cores is similar across wetlands. However, differences between sites are apparent below 10 – 20 cm depth, and in the N-rich wetlands studied (natural and urban tidal wetlands) they reflect N sources. In a more N transport-limited environment (urban nontidal wetland), N content below 20 cm depth shows spatial variation that could reflect either N usage or historical sediment composition from when the interior site was a pond.

Nitrogen isotopic composition of cores

N isotopes down cores reflect changing sources of N in both tidal wetlands and reflect usage in the nontidal wetland. Both tidal wetlands show δ^{15}N signatures down the cores that reflect the gradual decrease in septic sources of N that feed these wetlands as the Patuxent and Anacostia Rivers are cleaned up of pollution. In the nontidal wetland, δ^{15}N signatures down cores differ between inlet and interior sites and likely reflect the usage of N by plants near the inlet rather than past influxes of pond sediment, as the residence time of fertilizer N at the inlet of the wetland is high (Prestegaard, pers. comm.). As in the model by Cecchetti et al. (2020) done in a small-scale wetland (552 m^2 area; UMD golf course nontidal wetland is 18,750 m^2), plants take up N at the inlet site, leaving very little N left in the cores in the interior site. The δ^{15}N across both nontidal sites reflect the fertilizer inputs and atmospheric deposition, but the atmospheric signal is only evident in the interior site when N from fertilizer is taken up.

Chapter 5: Conclusions and Implications

Decomposition and vegetation experiments

The most obvious reservoir of carbon in a wetland is the vegetation itself. The peak biomass values do not exhibit clear trends among the wetland sites. Factors that determine peak biomass in freshwater wetlands are more complex than proximity to inlet and age or setting of the wetland. Vegetation diversity, however, appears to be related to wetland age and amount of disturbance, as aggressive species like *Typha latifolia* can easily take over a constructed wetland before native species can establish themselves once given a foothold.

Carbon and nitrogen losses from vegetation occur during decomposition of C and N and incorporation of biomass into marsh soils. Rates of C loss in the 8- to 9-week litter decomposition experiment were similar across the study wetlands. Carbon loss from the vegetation was not accompanied by isotopic changes, implying C loss by mechanisms such as leaching or tidal flushing rather than vegetation decomposition by microbial decay, at least in the early decomposition stages. The natural tidal wetland *Typha* had both higher initial C content and a slightly higher rate of C loss than observed in other wetlands. This suggests that leaching may preferentially remove non-structural C that *Typha* produces in higher amounts in favorable, nutrient-rich environments.

In the process of litter decomposition, nitrogen content does not consistently decrease over 8 – 9 weeks, while C does. N isotopes in fresh *Typha* appear to reflect the dominant sourcesof incoming N to the wetlands. Like the C isotopes results, N isotopes do not vary significantly with litter decomposition, implying that N is not lost in early stages of litter

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decomposition. Enrichment of ^{15}N in the upper cores compared to the decomposed vegetation in the tidal wetlands suggests that microbial processes (denitrification) could be important for N loss after 9 weeks of litter decay.

Evaluation of hypotheses

1. *Marsh sites with a higher value of peak primary productivity will sequester more C and N than other sites.*

   Peak biomass does not appear to follow clear trends across wetland sites. C and N sequestration is clearly higher in the natural tidal wetland, which does not have the highest biomass values. C and N stocks are higher in the interior of the nontidal wetland, yet the interior does not have a particularly peak high biomass. Peak biomass is clearly not the most important factor in determining C and N stocks – other factors like sediment dynamics during marsh formation may be more important.

2. *Marsh sites with faster* Typha *leaf and stem decomposition rates will sequester less C and N in marsh soils than other sites.*

   Typha decomposition rates are similar across sites. Initially, the natural tidal wetland has a slightly faster decomposition rate. However, the natural tidal wetland sequesters more C and N than the other sites although its decomposition rates are slightly faster. The difference may be due to the higher C content observed in the vegetation found in the natural tidal wetland.

3. *Sites in an established wetland will sequester more C and N than recently restored sites.*

   The natural tidal wetland consistently sequesters more C and N than the constructed urban wetlands. In addition, the natural tidal wetland has a higher % OM in core samples compared to the other wetlands. It is visibly organic down to 50 cm core depth and below. It is also far older than the urban tidal wetland (constructed 2010) and the urban nontidal wetland (constructed 2001).

4. *Marsh sites with the highest vegetation biodiversity will have the fastest* Typha *decomposition rates compared to other sites.*

   As described in Chapter 1, a higher vegetation biodiversity may translate to higher decomposition rates (Hector et al., 2000). In areas with no vegetation, soil microbes are not primed to decompose SOM as they are in areas with diverse types of vegetation (Kuzyakov et al., 2000; Mueller et al., 2016). While my study found that rates of C loss across wetland sites were similar, the natural tidal wetland had a slightly higher C loss rate than other sites in the first 2 weeks. The natural tidal interior site also has significantly higher biodiversity than the other sites, suggesting that microbial priming could be speeding up the decomposition rate. However, as C is not fractionated isotopically with C loss in the vegetation, it is likely that microbial priming is not an important factor in C loss in the study wetlands and that diversity may not affect decomposition rates as expected.

5. *Sites with seasonal to weekly intermittent wetting and drying cycles will exhibit the fastest* Typha *decomposition rates.*

Microbial decomposition has been observed to be faster in areas with season to weekly wetting and drying cycles (Battle and Golladay, 2001). The tidal wetlands (especially at inlet sites) exhibit daily wetting and drying cycles, while the nontidal wetland and the interiors of the tidal sites are more affected by ET and precipitation, but still receive frequent flooding events. ET varies seasonally, and precipitation-driven flood events occur less frequently than tidal cycles. As C loss rates from leaf packs are similar across all wetlands and is likely not microbially determined, it follows that microbial stimulation from wetting/drying cycles may not be an important factor in C removal from these freshwater wetlands.

However, intermittent wetting and drying may speed up leaching or tidal flushing. As rates of C loss are mostly similar across all three wetlands but only slightly faster in the first two weeks in the natural tidal wetland, all three study wetlands are likely to have enough waterflow for leaching of C to occur on the platforms.

Carbon stocks and core records of C and N

The three study wetlands' soils have distinct characteristics, primarily that the natural tidal wetland core consists of organic horizons until deeper than 50 cm, while the urban wetlands are only organic until 30 – 40 cm depth. The relationship between % OM and bulk density in the natural tidal wetland is more linear than the urban wetlands', which is evidence that the natural tidal wetland compacts more evenly because it is more homogenous.

While the natural tidal wetland is higher in OM and % C compared to the two urban wetlands, all three wetlands are relatively low in OM content relative to other studies done in freshwater wetlands. A low OM content implies that there are inputs of sediment that contain inorganic C into the wetlands. Inorganic C (as carbonates) may be a more important part of C storage in these freshwater wetlands, especially in the urban wetlands (Lorenz and Lal, 2015), than expected.

Generally, C storage is greater in older, natural wetlands, as expected. In the urban wetlands, spatial differences in C storage are more apparent because of differing sediment dynamics in construction of wetlands. Decomposition rates of *Typha* litter do not appear to affect carbon storage.

C is lost consistently with depth throughout the organic cores (the entire natural tidal core, and the upper portions of the urban cores), and $\delta^{13}C$ does not change significantly with depth. Carbon loss without fractionation suggests that leaching or flushing is more important at removing C from cores than microbial decomposition is, just like how leaching and flushing are important in decomposition of leaf litter.

The inorganic portions of the urban cores show varying $\delta^{13}C$ signals that imply mixing of organic C with two different types of inorganic C sources. In the urban tidal wetland, the source of inorganic C may be carbonate dust from a nearby cement plant. In the nontidal wetland, $\delta^{13}C$ values in the interior site are either due to pond sediment dynamics early in wetland formation, or to golf course management dumping C4 plant material near the marsh interior.

Nitrogen content and isotopic composition in the cores primarily reflects sources of N in the N-rich wetlands and reflects N usage in the sites limited by N because of lack of transport. In the N-rich wetlands, (both tidal wetlands) core % N is overall higher in wetlands with higher N loading (i.e. the natural tidal wetland), and % N shifts with depth reflect changes in N loading

over years. Core $\delta^{15}N$ reflects the isotopic signature of the dominant source of N. In the N-limited wetland site (i.e. the nontidal wetland interior), low values of % N below the rooting depth reflect either uptake of fertilizer N by plants near the inlet, or the effect of historical inputs of N-poor pond sediment to the interior. Depleted values of $\delta^{15}N$ could reflect uptake of fertilizer N by plants near the inlet, revealing the atmospheric N left in the interior site soil.

Evaluation of hypotheses

1. *Soil organic matter (SOM) content in the upper layer of the cores will be lower in areas with faster* Typha *decomposition rates.*

   C and OM loss rates throughout the litter experiments does not vary much among sites, although they are initially slightly faster in the natural tidal wetland. However, the OM content of the upper cores is higher in the natural tidal wetland. Rates of C loss in the vegetation do not greatly impact the OM in the upper cores.

2. *SOM content of the soil will be higher near the surface of the soil. I also hypothesize that the SOM content will increase in recalcitrance with depth in the soil profile and will therefore show higher C:N ratios deeper in the soil.*

   SOM content is indeed higher at the surface of all wetland sites than at depth. Cores exhibit similar C:N ratios throughout depth. The only exception to this (near base of nontidal wetland) involves transport of organic-rich pond sediment during marsh formation or dumping of C-rich plant material. It appears that C and N in soil cores is lost evenly with depth.

3. *C isotopic signatures near the surface of the soil profile will be similar to decomposed vegetation values but will change with depth up to the base of the roots (~20 cm). I also hypothesize that the ^{13}C content of soil from the sites with more diverse vegetation will be different from the ^{13}C content of soil from the most homogenous sites.*

C isotopic signatures do not change much with depth throughout the organic portion of wetland cores and are similar for each wetland. The only exceptions involve the inorganic parts of both urban cores, and they show that contamination by C from either carbonate dust (in the urban tidal wetland) or from pond sediment or C4 plant residue (in the urban nontidal wetland) can impact $\delta^{13}C$ values.

N isotopic signatures vary more with depth and across wetlands than $\delta^{13}C$ according to N sources and N usage, implying that while microbial decomposition is not very important in transforming C or N in cores, N is strongly affected by nutrient sources and usage, while C is primarily affected by sediment dynamics or large-scale, anthropogenic additions of C to sites.

4. *Core C content will reflect the effects of water fluxes at each site. Removal of DOC should cause a decrease in carbon content with relatively little change in carbon isotopic composition.*

As organic C decreases with depth without fractionation of C, removal of DOC by tidal flushing/leaching is clearly a major factor in C fluxes away from freshwater wetlands.

Nitrogen decreases with depth at all sites as well, but it isotopically shifts with depth. These isotopic shifts appear to follow trends based on changes in sources or uptake of

N, rather than denitrification or microbial transformations. However, the isotopic signals of microbial N transformations may be masked by changes in N sources, as denitrification is known to occur in the natural tidal wetland (Fowler, 2014), and may occur in both tidal wetlands as decaying leaf litter incorporates into the soil.

Implications and future work

Carbon is initially lost without apparent evidence of microbial decomposition throughout the wetlands in both the *Typha* litter and the organic parts of the soil cores across all wetlands. Removal of DOC with leaching/tidal flushing is a key flux of C from all three freshwater wetlands. Records of C content and isotopic signatures in the cores are also affected by the age of the wetland and, in constructed urban wetlands, sediment dynamics during wetland formation and anthropogenic inputs of carbonates, and less by biomass and biodiversity. In addition, most studies focus on organic carbon only when studying freshwater wetlands. As the three wetlands I studied have an unexpectedly low OM content and show evidence of contamination by carbonate dust, it follows that inorganic C (carbonates) may be an important factor in C storage in freshwater wetlands, particularly in constructed, urban ones.

Nitrogen dynamics are more complex than the C dynamics in these study wetlands. N content and isotopic signatures in both vegetation and cores are primarily affected by the incoming nitrogen – source type and load size. However, in sites with limited N transport, signals of N uptake by plants appears to impact core records. Microbial transformations of N may occur in all wetlands, but their isotopic signatures may be overshadowed by N source signatures. Evidence of denitrification particularly is visible in the tidal wetlands as *Typha* litter incorporates itself into the upper layer of the cores.

To study core records of carbon and nitrogen storage in freshwater wetlands, more work should be done examining the importance of inorganic C. While wetlands contain larger organic horizons than other ecohydrological settings, inorganic C appears to be a significant component of the urban wetland carbon reservoirs. Studies isolating rates of DOC removal compared to rates of microbial decomposition would help determine whether C removal in vegetation and cores is in fact dominated by DOC fluxes instead of microbial processes. Future work could also examine N dynamics in wetlands of varying ages and with various sources of N. As signals of N transformations may be masked in cores and vegetation by signals from N sources, future work could attempt to isolate isotopic signals of denitrification in tidal wetlands from N source or uptake signals.

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