Carbon cycling and storage in a temperate freshwater marsh in eastern Ontario

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Abstract

Freshwater marshes are underexplored yet significant carbon (C) sinks, playing a critical role in mitigating climate change. This thesis investigated C storage and cycling within a *Typha*-dominated freshwater marsh at the Mer Bleue wetland complex in eastern Ontario, Canada. Field sampling during the 2022 growing season was conducted to quantify C pools across above- and belowground biomass, soil, and water. The results demonstrated that belowground biomass is the largest C stock, containing seven times more C per unit area than aboveground biomass. Soil C stocks were also substantial, with the mineral soil layer contributing a significant proportion due to its high clay content. Seasonal trends in dissolved organic carbon (DOC) concentrations suggested minimal downstream transport, indicating effective retention within the marsh. Analyses of isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$-nitrogen) and C:N ratios revealed limited decomposition at depth, with the mineral soil layer showing potential for long-term C stabilization. Contrary to expectations, soil C:N and soil $\delta^{15}N$ did not consistently decrease with depth, highlighting unique decomposition and nitrogen dynamics in waterlogged soils. Additionally, the study underscored the need for refined bulk density measurements and integrated assessments of both organic and mineral soil layers to improve marsh C stock estimates. These findings contribute to the understanding of freshwater marsh ecosystems as dynamic C sinks, emphasizing their conservation and the importance of continued research to inform wetland management strategies under changing environmental conditions.

Resumé

Les marais d'eau douce sont des puits de carbone (C) peu étudiés mais significatifs, jouant un rôle crucial dans l'atténuation des changements climatiques. Cette thèse a examiné le stockage et le cycle du C dans un marais d'eau douce dominé par le *Typha*, situé dans le complexe de zones humides de la tourbière Mer Bleue, dans l'est de l'Ontario, au Canada. Des échantillonnages sur le terrain ont été réalisés pendant la saison de croissance 2022 pour quantifier les réservoirs de C dans la biomasse aérienne et souterraine, le sol et l'eau. Les résultats ont montré que la biomasse souterraine constitue le plus grand stock de C, contenant sept fois plus de C par unité de surface que la biomasse aérienne. Les stocks de C dans le sol étaient également importants, la couche de sol minéral contribuant de manière significative grâce à sa forte teneur en argile. Les tendances saisonnières des concentrations de C organique dissous (DOC) ont suggéré un transport en aval minimal, indiquant une rétention efficace au sein du marais. Les analyses des signatures isotopiques (δ^{13}C et δ^{15}N) et des rapports C:N ont révélé une décomposition limitée en profondeur, la couche de sol minéral montrant un potentiel de stabilisation à long terme du C. Contrairement aux attentes, les rapports C:N du sol et les valeurs de δ^{15}N du sol n’ont pas diminué de manière constante avec la profondeur, soulignant des dynamiques uniques de décomposition et d’azote dans les sols saturés en eau. De plus, l’étude a mis en évidence la nécessité d’affiner les mesures de densité apparente et d’adopter des évaluations intégrées des couches de sol organique et minéral pour améliorer les estimations des stocks de C des marais. Ces résultats contribuent à la compréhension des écosystèmes des marais d’eau douce en tant que puits de C dynamiques, soulignant leur importance pour la conservation et la nécessité de poursuivre les recherches afin d’orienter les stratégies de gestion des zones humides face aux changements environnementaux.

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Contribution of Authors

This thesis comprises an abstract, general introduction, literature review, original research chapter, general discussion, and general conclusion. The abstract provides a broad summary of the thesis. The general introduction contextualizes the research, highlighting its significance while introducing the research question, objectives, and hypothesis. The literature review situates the thesis within the current research on wetland carbon (C) storage, with a specific focus on marsh C storage and its movement through C pools (above- and belowground biomass, water, and soil). The original research chapter (Chapter 3) presents the results and findings, addressing how C moves and is stored within the C pools of a *Typha*-dominated freshwater marsh in eastern Ontario. The general discussion and conclusion summarize the findings, discuss the study's limitations, and outline broader implications and directions for future research.

With guidance from my supervisors, Drs. Cynthia Kallenbach and Ian Strachan, I developed the objectives, hypothesis, and research design. Field data collection was supported by my supervisor Dr. Ian Strachan and research assistant Ms. Josephine Messersmith. I conducted the majority of the laboratory work, with occasional technical assistance and support from undergraduate assistants. The isotope analysis for $\delta^{13}C$ and $\delta^{15}N$ was performed in the McKinney Lab by Ms. Hannah Lieberman. I was responsible for data collection, analysis, and the original writing of this thesis, with editorial feedback and guidance from my supervisors, Drs. Cynthia Kallenbach and Ian Strachan.

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List of abbreviations

Abbreviation	*Full description*
C	Carbon
CH_4	Methane
CO_2	Carbon Dioxide
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
MAOC	Mineral-Associated Organic Carbon
N	Nitrogen
NPP	Net Primary Productivity
POC	Particulate Organic Carbon
SOC	Soil Organic Carbon
TDN	Total Dissolved Nitrogen
$\delta^{13}C$	Carbon-13 Isotopic Signature
$\delta^{15}N$	Nitrogen-15 Isotopic Signature

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CHAPTER 1: General Introduction

Interest in carbon (C) sink ecosystems is growing, as rising levels of atmospheric C contribute to the increasing damage climate change inflicts on our environment (Loder & Finkelstein, 2020; Rydin et al., 2013; Villa & Bernal, 2018). Wetlands are particularly important in this context, covering only 5-8% of terrestrial landscapes yet storing ~ 500-750 Pg of the world's soil C, with one-third of this C stored in peatlands (Gorham, 1991; Mitsch et al., 2013).Wetlands accumulate C in part due to their flooded soils, which creates an anaerobic environment that limits microbial decomposition, thereby facilitating peat accumulation and reducing C loss to the atmosphere (Rydin et al., 2013).

Unfortunately, wetlands are vulnerable to anthropogenic land use change, and consequently may lose significant amounts of this stored C. These losses occur both indirectly, through climate change-induced environmental shifts, and directly, via land-use transformation (Hopple et al., 2020; Lin et al., 2022). Microbial activity and decomposition in wetlands increase with climate change induced annual temperatures increases and periods of drought (which turn previously anaerobic soils aerobic; Lasota & Błońska, 2021). Since European colonization in southern Ontario, 55.8% of the region's wetlands have been converted to other land use (equivalent to ~ 1 911 Tg C), with marshes experiencing the greatest losses at 88.9% (equivalent to ~ 1 600 Tg C; Byun et al., 2018). However, these estimates focus primarily on peat-based wetlands, potentially overlooking C loss from wetlands whose soils are categorised as mineral, such as freshwater marshes. Freshwater marshes—defined by their macrophytic plants (like *Typha* spp.), slow or stagnant waters, and predominantly mineral soils—store C in their above- and belowground biomass, water (as dissolved and particulate organic C), and soil (Bansal et al., 2019).

The underestimation of freshwater marsh C stores, combined with their vulnerability to land use change, underscores the need to quantify where C is stored within these systems to gauge what we stand to lose. C gas flux studies have already demonstrated that marshes intake significantly more atmospheric C than they release. For example, Strachan et al. (2015) estimated a long-term mean net annual C flux uptake of 97 ± 57 g C m^{-2} yr^{-1} at the Mer Bleue marsh in eastern Ontario. This raises the question: where is this C ending up, and could it be lost from the marsh in other ways besides C emissions? My thesis aims to address these questions, continuing the research at Mer Bleue by exploring how C is stored and cycled within a *Typha*-dominated freshwater marsh in eastern Ontario, focusing on the biomass, soil, and water of the system.

Typha generate substantial biomass, with peak growing season aboveground biomass at 1149 - 2600 g dry biomass m^{-2}, and their root mat belowground biomass at 811 – 2571 g dry biomass m^{-2} (Asaeda et al., 2008; Bonneville et al., 2008; Sharma et al., 2008). Due to *Typha*'s high lignin content, their organic matter breaks down slowly, allowing leaf litter to accumulate in the marsh (Li et al., 2019; Sharma et al., 2020). I expect the belowground biomass, which consists of both the live root mat and accumulated leaf litter and dead roots, to store the largest quantity of C, as also suggested by Strachan et al. (2015). Additionally, I foresee aboveground biomass C stores to be a significant temporary C sink depending on the growing season period. While plant inputs to the marsh likely represent a large source of accumulated C, much of this biomass is still vulnerable to slow decomposition by anaerobic microbes. During decomposition, mobile dissolved organic matter is produced, and some plant biomass is transformed into the soil organic matter pool. Whether these processes result in loss or storage pathways of marsh C remains uncertain.

Freshwater marsh soil C is understudied, limiting proper understanding of its C storage potential (Loder & Finkelstein, 2020). For instance, while marsh soils are traditionally categorized as solely mineral, many include a shallow organic layer with high C concentrations that range from 305 to 451 g C kg^{-1} dry soil, contributing to their overall storage capacity (Lasota & Błońska, 2021). Byun et al. (2018) estimate the organic layer to store 142 kg C m^{-2}. Yet, the deeper C reserves in the mineral layer are particularly intriguing, as most studies focus only on the top 30 cm of the soil profile (Loder & Finkelstein, 2020). C concentrations in the mineral soil layer range from 3.45 to 10.0 g C kg^{-1} dry soil (H. Wang et al., 2011; Zhang et al., 2008) which is higher than well drained soils (Gabriel et al., 2018). Mineral soils retain C through physiochemical bonds which reduces access for microbial decomposition (Goh, 2004), and may also offer important protection during periods of drought or rising temperatures (Gentsch et al., 2018). Considering the C accumulation and retention characteristics found in the organic and mineral soil layers, I expect the marsh's soil to be a significant C store.

In contrast to biomass and soil, dissolved organic carbon (DOC) in the water column represents a more volatile C store. DOC originates from decomposing organic matter and root exudates and is composed of less complex C molecules, making it more readily consumed by microbes or transported downstream (Bansal et al., 2019; Flanagan et al., 2006; Hensgens et al., 2020; Marx et al., 2017). These processes cause DOC concentrations to fluctuate throughout the growing season, generally falling within 40-80 mg C L^{-1} and peaking mid-growing season (Chu et al., 2015). Although net C gas flux studies suggest a net gain of C in marsh ecosystems, lateral movement of DOC could represent an important, yet underexplored, pathway of C loss. However, the slow water velocities in marshes cause high DOC and particulate organic C sedimentation rates of ~1.7×10^5 g C ha^{-1} yr^{-1} (Bridgham et al., 2006; Harter & Mitsch, 2003), which I expect to minimize downstream C loss.

Given the potential for marshes to act as significant C sinks, while also being under explored, it is critical to evaluate their C stores comprehensively. The objectives of my thesis are to quantify the different C pools in the marsh, characterize the degrees of decomposition within the marsh's C pools, and assess how these C pools and their degrees of decomposition change within the marsh spatially and over the growing season. I foresee that the biomass and soil will store significant amounts of C, with minimal decomposition due to the anaerobic environment. Furthermore, I expect the C pools to fluctuate over the growing season but not show a marked increase from upstream to downstream.

CHAPTER 2: Literature review

2.1 Freshwater temperate marshes in the context of climate change

With rising atmospheric carbon dioxide (CO_2) levels contributing to climate change, the role of wetland carbon (C) sinks has garnered increasing interest (Loder & Finkelstein, 2020; Rydin et al., 2013; Villa & Bernal, 2018). Globally, wetland ecosystems hold approximately 400-750 Pg C (Mitsch et al., 2013; Villa & Bernal, 2018). The substantial C storage in wetland ecosystems is, in part, due to their plant C uptake, through photosynthesis, surpassing their low decomposition rates, thereby inhibiting organic C from re-entering the atmosphere (Rydin et al., 2013).

Peatland wetlands are well documented for their substantial C stores in organic soil known as peat (an accumulation of incompletely decomposed organic material due to saturated soil conditions). In the Hudson Bay lowlands, peat deposits alone store ~30 Pg C (Harris et al., 2022). In contrast, marsh soils are mineral-based with smaller peat deposits than peatlands, if any (Mitsch & Gosselink, 2015; Rydin et al., 2013). Freshwater marsh C stores, compared to peatlands, are insufficiently documented and might constitute larger C sinks than previously thought. For instance, Byun et al. (2018) estimate marshes in southern Ontario contain 142 g C m^{-2}; however, these estimations solely account for peat deposits in marshes and overlook the C in the mineral soil layer.

Marshes exhibit high rates of net primary productivity (NPP), removing large quantities of atmospheric CO_2 and producing considerable amounts of plant biomass. This C sink is partially offset by marshes' substantial methane (CH_4) respiration. The global warming potential of CH_4 is 25 times greater than that of CO_2, though its atmospheric residency time is significantly lower at 12 years while CO_2 ranges from 1 to 173 years (Intergovernmental Panel on Climate Change, 2007).

Strachan et al. (2015) quantified the C gas fluxes (CO_2 and CH_4) of a marsh in the Mer Bleue wetland complex in eastern Ontario over the course of five years, where they observed an average net uptake of 97 ± 57 g C m^{-2} yr^{-1}. Despite wetlands' ability to potentially uptake and retain C, they are at risk of land use conversion that drains these ecosystems of water, inducing stored C to be respired from their soils (Lin et al., 2022).

In southern Ontario, 88.9% of freshwater marshes have been converted to support agriculture and urban infrastructure (e.g. drains, roads, and buildings), resulting in an estimated loss of 1600 Tg C of marsh C stocks (Byun et al., 2018). The alteration of marsh ecosystems entails the loss of both established C stores and potential future C sequestration. These concerns underscore the importance of properly quantifying the C pools in freshwater marshes, understanding how C moves within the system and the degree of its retention.

2.2 Freshwater temperate marsh classification

Freshwater marshes are minerotrophic wetland ecosystems characterised by a shallow and slow-moving or standing water regime, mineral soil matrix with little to no peat deposits, and vegetation composed of macrophytes such as cattails (*Typha* spp.) (Mitsch & Gosselink, 2015; Rydin et al., 2013). In a temperate freshwater marsh, C is stored in plant biomass, soil, and water (Fig. 2.1), but the distribution and potential residence time of C among these storage pools has been poorly characterized.

The seasonality of temperate climates influences the C stores and processes of marshes, notably NPP and decomposition are high in summer and low in winter (Atkins et al., 2017;

Bonneville et al., 2008). Additionally, seasonal variations like precipitation and spring snowmelt affect marsh hydrology, influencing streamflow and, consequently, dissolved organic C (DOC) concentrations (Ågren et al., 2010; Boyer et al., 2000).

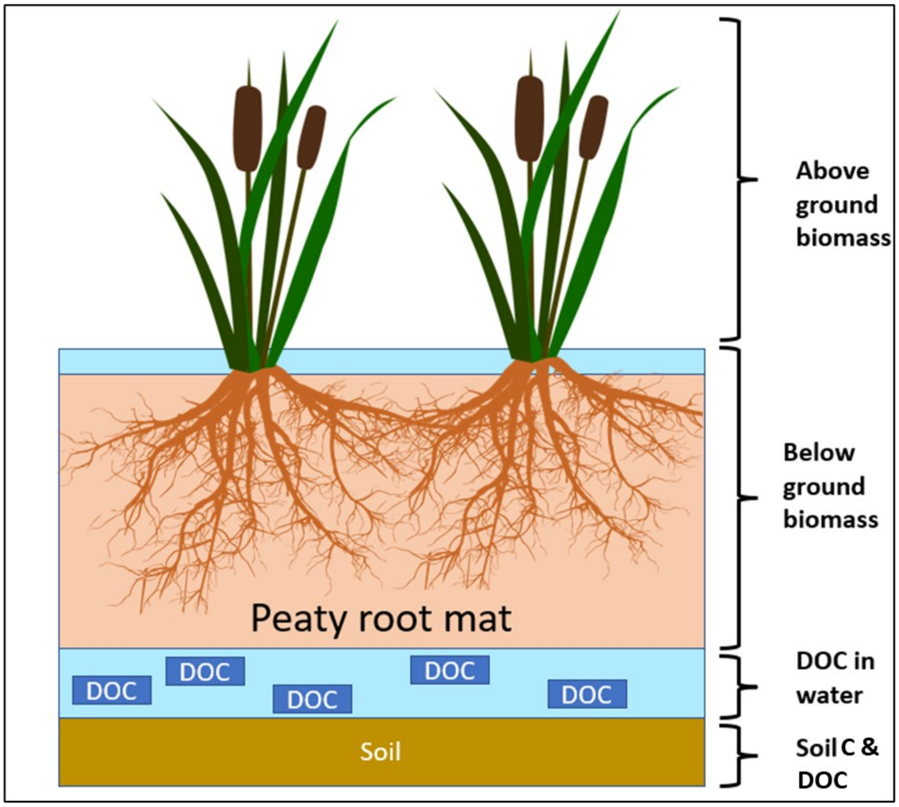

Figure 2.1 Carbon pools within a freshwater temperate marsh; dissolved organic carbon (DOC) (adapted from Bansal et al., 2019).

2.3 Carbon pools in a freshwater marsh

In this section, I investigate the literature pertaining to the C storage and movement within freshwater temperate marshes dominated by cattails (*Typha angustifolia L.*). More specifically, I utilise this study's field site, the Mer Bleue marsh in eastern Ontario, Canada, as a framework to explore these questions.

2.3.1 *Typha* biomass

Typha dominate hydrologically disturbed wetlands, such as marshes, where their adaptations (e.g., flood and drought tolerance) give them an advantage over other native plant species and allow them to grow in dense monocultures (Bansal et al., 2019). *Typha*'s biomass comprises aboveground components (stems, leaves, and a reproductive organ) and belowground components (roots and rhizomes) (Asaeda et al., 2008). Additionally, the belowground biomass (also referred to as the root mat) accumulates both above and belowground detritus biomass (Fig. 2.1).

The rhizomes store carbohydrates that fuel emerging shoot growth in late spring (Asaeda et al., 2008; Bonneville et al., 2008). During the growing season, *Typha* exhibit high rates of NPP, peaking in late spring and early summer (Asaeda et al., 2008; Bonneville et al., 2008; Chapin & Eviner, 2014). Their aboveground biomass reaches critical mass in mid-August at 1149 - 2600 g dry biomass m^{-2} (Asaeda et al., 2008; Bonneville et al., 2008). Once shoots are established, *Typha* allocates C back into the rhizomes until the shoots senesce in October (Asaeda et al., 2008; Bonneville et al., 2008). Eventually, environmental forces such as wind and snow push the senesced shoots over into the water and root mat, subsequently accumulating as leaf litter.

Rhizomes persist year-round with a turnover rate of ~2 years, while that of roots is every ~8 weeks (Sharma et al., 2008). Upon senescence, rhizomes and roots accumulate as root litter in the water, decomposing into particulate organic C (POC) and DOC (Bansal et al., 2019). The rhizome’s mass reduces slowly during the winter and then more quickly in the spring when they translocate C up to new shoot growth (Sharma et al., 2008). Sharma et al. (2008) observed that the belowground biomass of *Typha angustifolia* is lowest in May (148 - 786 g of dry biomass m^{-2}) and highest in August at (811 – 2571 g of dry biomass m^{-2}).

Typha accumulates and stores significant amounts of C in its biomass, however, a portion of the assimilated C is lost through root exudation. Plant roots secrete 10-30 % of their NPP as small, low molecular weight C compounds into the water through root exudates (Chapin & Eviner, 2014). These root exudates, comprised of water-soluble organic compounds such as sugars, amino acids, and organic acids, contribute to the DOC in the water column and soil and are a primary microbial energy source, fueling the metabolism of the marsh microbial community (Reddy & DeLaune, 2008; Waldo et al., 2019).

2.3.2 Water and soil dissolved organic carbon

C exists within the water column and pore soil water of the marsh as DOC and POC, where DOC refers to particles <0.45 µm (Bansal et al., 2019; Boyer et al., 2000; Herbert & Bertsch, 1995). DOC sources include decomposing plant matter, root exudates, and upstream imports (Bansal et al., 2019; Hensgens et al., 2020; Marx et al., 2017). DOC can undergo rapid transport out of the marsh through mineralization to CO_2 or exported downstream (Ågren et al., 2010; Flanagan et al., 2006; Sokol et al., 2019). Higher water inflow rates upstream from the marsh can dilute DOC concentrations and increase fluxes, mobilizing DOC and increasing DOC exports (Andersson & Nyberg, 2008). DOC production rates within the wetland water column can be high, resulting in sometimes similar DOC and total organic C concentrations (Laudon et al., 2004). This trend is not necessarily seen in the soil pore water where in the wetlands in northeastern China, Xi et al. (2007) observed the proportion of DOC to total organic C (DOC plus POC) to average 67.5%.

The water regimes of temperate freshwater marshes are generally shallow and slow-moving, excluding during significant water influx events (such as major rain events or during spring snowmelt) which increases the water discharge (Boyer et al., 2000). These water events dilute marsh water, thereby decreasing water DOC concentrations and resulting in early spring DOC concentrations often lower than the rest of the year. As the growing season continues and the snowmelt water influx subsides, combined with the growth of hydrophytes and more rapid decomposition, DOC concentrations increase. Chu et al. (2015) reported yearly marsh DOC concentrations ranging from 40-80 mg C L^{-1} and peaking in May and June. Additionally, they observed on average a net DOC and POC flux into the marsh of ~23 Cg m^{-2} yr^{-1}.

The low water velocity of marshes results in an approximate sedimentation rate of 1.7×10^5 g C ha^{-1} yr^{-1} (DOC and POC) accumulating in the soil (Bridgham et al., 2006; Harter & Mitsch, 2003). Liu et al. (2014) reported that the DOC in marsh mineral soil during the growing season ranged from 0.36-0.58 mg C g^{-1} dry soil. DOC's light molecular makeup makes it preferential for microbial consumption, leading to DOC mineralization and outgassing as CO_2 and CH_4 (Boddy et al., 2007). However, DOC is also readily sorbed onto mineral surfaces of marsh sediment in the soil profile through abiotic processes (Kothawala et al., 2012). The sorption of DOC to mineral surfaces contributes to its protection from further microbial decomposition and thus can be a possible mechanism for C storage in the marsh soil profile. This immobilization of C in the marsh's soil may prevent DOC mineralization further downstream in ecosystems where DOC may more easily decompose. Estimates of how much DOC enters the soil system in freshwater marshes through this pathway of sorption have not been quantified. In the wetland soils of the Yellow River Delta, in northeastern China, Zhao et al. (2020) estimate that DOC contributes to less than 0.5% of the total soil organic C. However, because sorption of DOC to mineral surfaces abiotically protects it from microbial attack, this fraction of immobilized C may be less sensitive to losses if the wetland is drained and thus could represent a pool of C relatively more stable to wetland disturbances.

2.3.3 Soil organic carbon

Globally, soils are the largest terrestrial organic C pools; however, marsh soil C stores are insufficiently documented and, potentially, underestimated (Loder & Finkelstein, 2020; Stockmann et al., 2013). Loder and Finkelstein (2020) attribute this research gap to marsh soils being categorized as mineral and not as a combination of mineral and organic, thus overlooking the C stores in their organic layer, and that marsh mineral soil C content is often only quantified in the top 30-40 cm. Loder and Finkelstein (2020) estimate that the short-term and long-term rates of soil C accumulation in marsh soils are 150 ± 87 and 28 ± 5 g C m^{-2} y^{-1}, respectively.

Soil organic C (SOC) originates from decomposing organic matter and within the soil profile takes the forms of DOC, POC, and mineral-associated organic C (MAOC). Soil C in the POC fraction consists of plant material under various stages of decomposition, with a large proportion only minimally decomposed in marshes because of the water-saturated conditions that limit microbial metabolism. MAOC represents C that has sorbed onto the mineral soil matrix, rendering it less available to microbes and resistant to decomposition (Emde et al., 2022; Mirabito & Chambers, 2023).

As organic material undergoes biotic and abiotic decomposition processes, more C is typically lost (as CO_2) relative to nitrogen (N). Thus, deeper in the soil profile, where organic matter has likely been more exposed to decomposition processes, the soil C:N is often lower compared to surface organic matter (White, 2005). The soil C:N, representative of the degree of decomposition, is thus sometimes used to indicate ‘older’ versus ‘newer’ organic matter.

However, this trend in a declining C:N with soil depth has primarily been shown in well-drained soil and is not necessarily consistent in poorly drained soils, like in marshes, where C:N can remain invariable with depth or even increase (Hornibrook et al., 2000; Liu et al., 2023; White, 2005). Hornibrook et al. (2000) observed the soil C:N at the Point Peelee Marsh to range from 12.0 – 17.0, decreasing with depth but stabilizing at ~20 cm, differing from the nearby bog that exhibited continual C:N decrease with depth. Liu et al. (2023) observed larger C:N variation with less linearity within the soil profile of the reed wetland in China, ranging from 9 - 99, increasing, decreasing, or consistent by depth depending on the sampling site. These trends complicate identifying decomposition trends through the soil profile but can be clarified by also observing changes in C and N isotopes (§2.3.5).

Organic layer

In marshes, the substantial quantities of organic matter produced by macrophytes (like *Typha*) are not easily decomposed and accumulate in the water, thereby facilitating anaerobic environments and further limiting decomposition (Bansal et al., 2019; Li et al., 2019). This organic matter accumulates in marshes as organic soils (or peat) at depths of 0.4-3.5 m (Byun et al., 2018). The Canadian System of Soil Classification subdivides wetland organic soils by the levels of decomposition as fibric (origin of the material is easily identifiable), mesic (intermediate stage of decomposition), and humic (advanced stage of decomposition) (Kroetsch et al., 2011). These organic soils exist as layers within the soil profile (where decomposition increases with depth) and exhibit C concentrations ranging from 305 to 451 g C kg^{-1} dry soil, where C concentrations negatively correlate with the extent of decomposition (Lasota &

Błońska, 2021). Byun et al. (2018) estimate that peat in marsh soils stores approximately 142 kg C m^{-2}.

While these peat soils have high C content, the absence of mineral soil in this layer means that C exists solely as POC, rendering it vulnerable to loss through decomposition or lateral movement downstream (Boyer et al., 2000). Moreover, disturbances like climate change and wetland draining increase decomposition rates and C loss in organic wetland soils by raising temperatures and converting the environment from anaerobic to aerobic (Harris et al., 2022; Hopple et al., 2020). Lasota and Błońska (2021) recorded 50% less C in drained organic soils compared to non-drained, due to increased C mineralization.

Mineral layer

Wetland mineral soil differs from the organic soil layer, as its composition allows for interactions between ions, microbial communities, and organic matter. Mineral soils are composed of varying ratios of sand, silt, and clay, and contain less than 20-30% organic matter which includes DOC, POC, and MOAC (Emde et al., 2022; Mitsch & Gosselink, 2015; Vepraskas & Craft, 2016). The Mer Bleue marsh formed in the abandoned river channel of the Ottawa clay plains, giving its mineral soil layer a high clay content (Geological Survey of Canada, 1987; Ministry of Agriculture and Food Ontario, 1987). In general, freshwater marsh mineral SOC ranges from 3.45 to 10.0 g C kg^{-1} dry soil (H. Wang et al., 2011; Zhang et al., 2008), which is much lower than that of Canadian organic soils but higher than many temperate well-drained soils (Gabriel et al., 2018).

The mineral soil layer above the parent material in the Ottawa clay plain (where the Mer Bleue Wetland complex is located) is up to 2 m in depth above the parent material, meaning an overall larger volume of soil compared to the organic layer (Ministry of Agriculture and Food Ontario, 1987). With this greater volume comes the possibility of the total SOC in the mineral soil layer being equivalent to or even surpassing that of the smaller organic soil layer.

Within the mineral layer, SOC is immobilised through both physical and chemical protection and stabilization and separation from microbial activity (Goh, 2004). The first physical barrier occurs through sediment deposition which can limit the amount of C in mobile forms (such as DOC and POC) suspended in the water column (Boyer et al., 2000; Sokol et al., 2019). This barrier isolates POC and DOC from microbial communities in the water column, where they are typically more active because of the higher concentrations of dissolved oxygen (Hedges & Keil, 1995). From here, POC and DOC deposition is further stabilized through physicochemical interactions with the mineral matrix where C sorbs to soil particles, transforming it into MAOC (Goh, 2004). In wetland soils, MAOC accounts for 7 - 91% of SOC (Liu et al., 2023). This transformation occurs through ligand exchanges, cation bridging, hydrogen bonding, and van der Waals forces between C molecules and mineral surfaces (Goh, 2004; Sokol et al., 2019). Soils with higher % clay exhibit higher C concentrations due to clay's higher cation exchange capacity, surface charge, and surface area (Bi et al., 2023). With depth, microbial activity decreases and so also does the likelihood of C mineralization, where the global microbial biomass C decreases from 16.7 Pg C in the 0-30 cm soil layer to 6.5 Pg C in the 30-100 cm soil layer (Sokol et al., 2019; Xu et al., 2013).

Mineral soils, such as those found in the bottom soil profile of marshes, may store less C per g of soil, but because of the above protection mechanisms and possibly larger total volume, have the potential to mitigate C loss during periods of droughts or drainage caused by land use change through physiochemical stabilization (Gentsch et al., 2018).

2.3.4 Microbiome, decomposition, and C stabilization

Organic matter undergoes various processes and transformations, including photooxidation, leaching, depolymerization, and mineralization (Chapin et al., 2011; Raza et al., 2023). However, mineralization through microbial activity is limited by the anaerobic conditions in the subsurface zones of wetlands, a consequence of water-logged organic matter accumulation. Despite these conditions, microbial activities in marshes are considerably higher compared to more acidic wetlands like peatlands (Mitsch & Gosselink, 2015). Microbial C processing efficiency ranges from 20-60% in aerobic conditions and 5-10% in anaerobic conditions (Adingo et al., 2021; Reddy & DeLaune, 2008). Although microbial biomass accounts for only 2% of the total organic C found in marshes (4 – 6% of SOC), its impact on C cycling through the decomposition of organic matter, and the mineralization and immobilization of C, is significant (Li et al., 2023; Reddy & DeLaune, 2008). However, microbial activity and the mechanisms governing decomposition in marshes remain understudied (Yarwood, 2018).

In freshwater marshes, decomposition initiates once the *Typha* senesces, with fungi and aerobic bacteria communities hydrolyzing complex, larger polymers into POC (Buesing & Gessner, 2006; Mitsch & Gosselink, 2015). Microorganisms continue breaking down leaf and root litter into smaller POC and DOC. However, due to the microbial growth constraints induced by oxygen limitations to metabolism, complete POC decomposition is limited such that much of the remaining partially decomposed biomass accumulates as peat (Van der Valk, 2012).

Fermentative bacteria and methanogens, both anaerobic microbes, play crucial roles in mineralizing POC and DOC into CH_4 (Van der Valk, 2012). Fermentative bacteria break down large organic compounds into simpler forms such as acetate, while methanogens use these compounds as terminal electron acceptors to complete methanogenesis (Garcia, 1990; Lyu et al., 2018; Van der Valk, 2012). Root exudates contribute to labile C pools and are associated with heightened CH_4 production (Bansal et al., 2019). They provide microorganisms with bioavailable C, increasing mineralization and decreasing soil organic matter (Yarwood, 2018). In a marsh in northeastern China, Liu et al. (2014) found that soil methanogen archaea populations correlated with soil DOC, and peaked in autumn, potentially impacting decomposition rates, leaf litter, or DOC (Liu et al., 2014).

Typha's aerenchyma, which aerates its root mat, also serves as a conduit for transporting microbial-produced CH_4 from below the surface into the atmosphere (Bansal et al., 2019; Van der Valk, 2012). Most CH_4 emissions from decomposition are released into the atmosphere, but a portion of the CH_4 passes through biofilms in oxygenated zones on *Typha* roots created by air pumped down through the plant's aerenchyma tissues. Approximately one-third of microorganisms in these zones are aerobic methanotrophic bacteria that metabolize CH_4, oxidizing it to CO_2 (Faußer et al., 2012). In a freshwater marsh dominated by *Typha latifolia* in Maine, U.S.A, King (1996) observed that methanotrophs consumed 1-58% of the CH_4 produced.

In addition to C molecules, organic N molecules are broken down by microbes. N is essential for the microbial community to process organic matter, as it fuels their protein synthesis, enzyme activity, and energy production (Paul, 2015). Once microbe N demands are met, microbes convert excess N into ammonium (ammonification), which also mineralizes C into CO_2 or CH_4 and reduces the amount of C relative to N in the substrate (Paul, 2015; Reddy & DeLaune, 2008). It is important to note that microbial enzymatic hydrolysis is often the limiting factor in these processes in anaerobic environments (Puyuelo et al., 2011). The result of these processes is reflected in the soil C:N, which decreases with increasing levels of decomposition, although it should be noted that this occurs more consistently during the earlier stages of decomposition (Chapin et al., 2011; Mirabito & Chambers, 2023; Reddy & DeLaune, 2008).

Anaerobic decomposition is optimal when organic matter has a C:N of 20 to 30, but is also influenced by the organic material chemistry (Puyuelo et al., 2011). The size and chemical bonds of C molecules influence the ease with which microorganisms process organic matter (e.g., sugars are more quickly processed than lignin), resulting in decreasing decomposition rates as more digestible C molecules are consumed from the C pool (Chapin et al., 2011; Ogata et al., 2002). The high lignin content in *Typha angustifolia*'s above and belowground biomass is 194 and 178 mg g^{-1} dry biomass respectively, and slows their decomposition (Li et al., 2019; Sharma et al., 2020). The placement of C in its environment also affects its likelihood of being mineralized. For instance, DOC, which exists freely in the water and soil matrix, is readily physically accessible for microbial consumption, while MAOC is not. Importantly, while rates of microbial C assimilation can be greater than the direct mineral sorption of plant-derived DOC, the microbial biomass C is itself an important contribution to MAOC. As microbial cells senesce, their microbial biomass and exudates easily sorb to clay and mineral surfaces, protecting these microbial-derived compounds from further decomposition (Kallenbach et al., 2015; Sokol et al., 2019).

The contribution of dead microbial biomass in the MAOC fraction is estimated at ~50 % (Angst et al., 2021) in aerated mineral soils where decomposition rates are rapid. In wetlands, including fresh-water marshes, the proportion of microbial biomass accumulating in MAOC is expected to be lower because of more limited microbial biomass production (Duan et al., 2024), but might still be a relevant source of accumulated minerally protected C. Microbial derived C accounts for 29.48% of SOC in wetland soil (Cao et al., 2023). Thus, in the process of microbial assimilation of more bioavailable marsh C such as DOC and new plant inputs, a proportion of that assimilated C may end up and accumulate in the more stable MAOC pool.

2.3.5 Isotopes as indicators of carbon and nitrogen cycling

Observing the natural abundance of isotopes can provide insights into the movement of C and N in an ecosystem (Krüger et al., 2023; Staddon, 2004). The $\delta^{13}C$ and $\delta^{15}N$ signatures are influenced by various processes (such as photosynthesis and decomposition), which cause fractionation and result in different isotopic enrichments in the C and N pools found in marshes (Hobbie & Högberg, 2012; Krüger et al., 2023).

Typha is classified as a C_3 plant, referring to the enzymatic pathway these plants utilize during photosynthesis, which results in their roots being equal to or $\delta^{13}C$-enriched compared to their leaves (Constable et al., 1992; Edwards & Still, 2008; Hobbie & Werner, 2004). Typically, C_3 plants are more $\delta^{13}C$ depleted than C_4 plants due to their stomatal discrimination against heavier C isotopes during photosynthesis (Hobbie & Werner, 2004; Krüger et al., 2023). *Typha*'s $\delta^{13}C$ signature is -27.7 to -24.1 ‰ (Hornibrook et al., 2000; Zheng et al., 2019). Different plant materials also exhibit varying ^{13}C enrichments, where simpler C molecules such as cellulose and hemicellulose are ^{13}C enriched, while lignin is more depleted (Krüger et al., 2023).

The $\delta^{13}C$ and $\delta^{15}N$ values typically increase with the degree of decomposition, leading to increased ^{13}C and ^{15}N enrichment with soil depth as deeper soil organic matter is often more decomposed, although this trend may vary depending on whether the soil is dominated by C_3 or C_4 plant cover (Hobbie & Högberg, 2012; Krüger et al., 2023). The $\delta^{13}C$ value of soil organic matter exceeds that of the plant itself because as the senesced plant is decomposed, the microbes will preferentially respire more ^{12}C, leaving behind relatively more ^{13}C in the plant litter and in their own microbial biomass (Staddon, 2004). Hornibrook et al. (2000) observed an increase in $\delta^{13}C$ ‰ values in the 0-20 cm layer of marsh organic soil profiles at Pointe Pelee (dominated by C_3 plants), ranging from -29.7 to -25.0, with consistent enrichment levels with increasing depth. In a forested wetland in North Carolina, USA, Minick et al. (2019) reported the $\delta^{13}C$ ‰ values increased with depth in the organic peat layer (ranging from -29.8 to -29.1) and mineral layer (ranging from -28.98 to -28.79) of the soil horizons. In this same study, SOC is negatively correlated with $\delta^{13}C$ in the soil, following the typical pattern with decomposition, a pattern that is not observed in the few studies that report the total N and $\delta^{15}N$ in marsh and mineral soil wetland ecosystems.

The estimated $\delta^{15}N$ of *Typha* biomass is approximately –5.2‰ (Dean & Biesboer, 1986). When used as a tracer in the plant, the roots and rhizomes are more enriched in $\delta^{15}N$ than their stems (Zheng et al., 2019), which are less enriched than the soils. However, due to the complexity of N pathways through the ecosystem, ^{15}N will not necessarily increase or decrease linearly when observing increasing stages of decomposition. Fractionation of ^{15}N in ecosystem processes can increase or decrease values or even do both simultaneously. For instance, nitrification, denitrification, ammonia volatilisation, and enzymatic hydrolysis cause ^{15}N enrichment (Handley et al., 1999; Hobbie & Colpaert, 2003; Högberg, 1997; Pörtl et al., 2007; Shearer & Kohl, 1986), while ion exchanges lead to depletion (Hubner, 1986). Processes like N_2 fixation, microbial N assimilation, and N mineralisation can result in either enrichment or depletion (Högberg, 1997; Kendall, 1998). Delwiche and Steyn (1970) observed this nonlinear trend through soil profiles in California, where $\delta^{15}N$ increased from 0.00122 to 0.00166 in the first 20.0 cm and then decreased to 0.0075 from 20.0 – 100.0 cm depth. The $\delta^{15}N$ in organic soils appears to follow a more similar trend to the $\delta^{13}C$, where it increases with depth. This is seen in a minerotrophic peatland in Alberta, Canada where Choi et al. (2007) report δ^{15}N increasing from 0.6 – 2.4 ‰ within a 30 cm of peat. By contrast, in a freshwater swamp (a mineral soil wetland) in Maryland, U.S.A, Elliott and Brush (2006) reported a δ^{15}N decrease from 6.9‰ to 1.7‰ with depth. However, due to the limited reporting on δ^{15}N in poorly drained soil systems, further exploration of δ^{15}N depth patterns across wetlands is warranted.

2.4 Importance of understanding carbon stores and movement in freshwater marshes

Freshwater temperate marshes play a crucial role in the global C cycle and may serve as significant C sinks with the potential to mitigate climate change impacts. For instance, the mineral soil may contain deep C stores that are often overlooked in many wetland C studies and offer protection to stored C, which the peat layer lacks. Through this literature review, I have explored various aspects of C dynamics and stores within these ecosystems, though some gaps persist, especially about DOC, MAOC, and organic matter isotopic values. Many of the C values referenced in this study were drawn from research on ecosystems similar to freshwater marshes, due to the limited availability of studies specifically focused on freshwater marsh C dynamics. While these estimates provide useful insights into freshwater C stocks, studies of the ecosystem itself are crucial for accurately understanding its role in the C cycle and its C storage capacity. My research addresses this gap through a field experiment at the Mer Bleue wetland complex, situated east of Ottawa, Ontario Canada, where I sampled *Typha* above and belowground biomass, water, and soil throughout the 2022 growing year. In analysing the C and N levels in the stores, as well as the natural abundance of δ^{13}C and δ^{15}N, I aimed to:

1. Quantify the C stores of the above and below biomass, water, and soil;

2. Use C:N and levels of $\delta^{13}C$ and $\delta^{15}N$ to delineate levels of decomposition and C turnover in the system; and,
3. Examine spatial trends in the C stores to discern potential losses from the system downstream.

CHAPTER 3:

Carbon cycling and storage in a temperate freshwater marsh in eastern Ontario

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Abstract

Rising atmospheric carbon (C) levels significantly contribute to climate change, increasing the focus on ecosystem C sinks such as wetlands. Many studies on wetland C sinks primarily address peatlands (organic soil wetlands), often overlooking the potential C storage provided by mineral soil wetlands like marshes. This study investigates C dynamics and storage in a freshwater temperate marsh dominated by *Typha*, with a focus on seasonal variation in C pools, decomposition, and retention across plant biomass and soil layers. Quantification of *Typha* biomass revealed that belowground biomass was the largest C stock, containing seven times more C than aboveground biomass. Soil C stocks were also significant, with the mineral soil layer exhibiting higher C concentrations than previously reported. Despite the organic layer having greater C concentrations, mineral soil also contributed nearly twice as much C than the organic layer when the relatively larger depth of the mineral layer is considered. Soil isotopic signatures ($\delta^{13}C$, $\delta^{15}N$-nitrogen) and C:N in soil layers revealed limited decomposition at depth, which may suggest long-term stability of soil C storage. This study highlights the importance of considering both organic and mineral soil layers in C stock assessments. Furthermore, dissolved organic carbon (DOC) retention within *Typha* stands suggested minimal downstream C loss, enhancing the marsh's role as a C sink. These findings emphasize the importance of integrating

organic and mineral soil layers in marsh C stock assessments and underscore the need for further research on the dynamics of mineral soil C and seasonal variability in marsh ecosystems.

3.1 Introduction

Wetland ecosystems are recognized for their carbon (C) storage and sequestration. Globally, these systems store ~400-750 Pg C and sequester ~0.83 Pg C of carbon dioxide (CO_2) annually, despite covering only 5-8% of terrestrial landscapes (Loder & Finkelstein, 2020; Mitsch et al., 2013; Rydin et al., 2013; Villa & Bernal, 2018). These C capturing capabilities are in part attributed to the anaerobic conditions of the saturated wetland soils which inhibit decomposition and lead to peat accumulation (Rydin et al., 2013). In Canada, approximately 19% of the land cover is classified as wetlands, giving the country significant potential for C storage (Mahdianpari et al., 2020). The Hudson Bay lowlands alone contain 30 Pg of C in their deep peat deposits (Harris et al., 2022). However, wetlands have historically been undervalued, leading to widespread drainage and land use conversion and causing massive soil organic C (SOC) losses (Lin et al., 2022). Byun et al. (2018) estimate that southern Ontario has lost 55.8% of its wetlands, resulting in the release ~ 1,911 Tg C stored in wetland ecosystems since European settlement. Marshes have been particularly affected by land use change, with only 11.1% remaining, leading to a loss of ~ 1,600 Tg C.

Wetland loss is driven not only by landscape conversion but also by other anthropogenic effects such as climate change, which causes droughts that alter wetland water levels and increase annual temperature averages (Harris et al., 2022; Hopple et al., 2020). These changes aerate the soil and stimulate microbial activity, which accelerates peat decomposition and wetland C losses up to 50% (Lasota & Błońska, 2021). Many wetland C storage studies focus solely on peat deposits, overlooking other potential C stores, (Byun et al., 2018) such as within the vegetation and mineral soil, thus underestimating the C sequestration potential of non-peatland wetlands, such as swamps and marshes.

Marshes (minerotrophic wetlands with little to no peat deposits) store significant C in plant biomass (above and belowground), the water column as dissolved organic C (DOC) and particulate organic C (POC), and in soil organic C (SOC). Macrophytes in these systems, such as cattails (*Typha* spp.), produce considerable biomass with elevated lignin content, making their organic matter more resistant to microbial decomposition (Bansal et al., 2019). During peak biomass production, *Typha* represent 1149 - 2600 g m^{-2} and 811 – 2571 g m^{-2} for above and belowground dry biomass, respectively (Asaeda et al., 2008; Bonneville et al., 2008; Sharma et al., 2008). Both decomposing plant biomass and root exudates contribute to DOC production, with DOC concentrations fluctuating throughout the year due to biomass production being confined to the growing season and seasonal temperature changes limiting microbial activity. Chu et al. (2015) reported yearly marsh DOC concentrations in the water column to range from 40-80 mg C L^{-1}. The shallow, standing to slow-moving water regimes in marshes promote C deposition (~1.7×10^5 g C ha^{-1} yr^{-1} of both DOC and POC) in the soil while limiting lateral transport downstream (Bridgham et al., 2006; Harter & Mitsch, 2003; Mitsch & Gosselink, 2015; Rydin et al., 2013). Marsh soil DOC during the growing season typically ranges from 0.36-0.58 mg C g^{-1} dry soil, contributing to less than 0.5% of SOC (Liu et al., 2014; Zhao et al., 2020).

The detritus from marsh vegetation accumulates in the organic layer of marsh soils at depths of 0.4-3.5 m, with C concentrations of 305 to 451 g C kg^{-1} dry soil (Byun et al., 2018; Lasota & Błońska, 2021). Below this layer, the mineral soil layer immobilizes C through physiochemical bonds, further inhibiting microbial decomposition and resulting in SOC ranging from 3.45 to 10.0 g C kg^{-1} dry soil (Goh, 2004; H. Wang et al., 2011; Zhang et al., 2008), lower than the peat layer but higher than many temperate well-drained soils (Gabriel et al., 2018). However, these mineral soil organic C (SOC) stores are insufficiently documented and, potentially, underestimated (Loder & Finkelstein, 2020; Stockmann et al., 2013). Additionally, the mineral soil's physiochemical bonds help SOC resist mineralization during anthropogenic disturbances (Gentsch et al., 2018; Goh, 2004; Sokol et al., 2019). Nevertheless, mineral soils are not immune to C loss and can release C when subjected to drying and rising temperatures (Gentsch et al., 2018), underscoring the importance of wetland conservation. When examining peat C deposits, Byun et al. (2018) estimate marsh soils store ~142 kg C m^{-2}, though this should increase when taking other pools into account.

Previous studies on short-term C accumulation in marshes provide insight into marsh C storage by measuring the net C entering and exiting the ecosystem. For example, over a seventeen-year period, the bog portion of the Mer Bleue Wetland Complex in Eastern Ontario, Canada exhibited a net C flux of -67 ± 44 g C m^2 yr^{-1} (Nugent et al., 2018). In comparison, the marsh portion of the same site has shown a net C flux of -97 ± 57 g C m^2 yr^{-1} over a five-year period (Strachan et al., 2015). While these figures only account for gaseous C exchange, they indicate that marshes, like peatlands, can absorb significant amounts of C, despite containing less C in their organic matter layer than peatlands.

We must quantify the freshwater marsh C stores to know what their potential for C loss in response to land use and global change. In this study we investigated how C is stored and cycled within a *Typha* dominated freshwater marsh in eastern Ontario. Our objectives were to: 1) quantify the different C pools in the marsh; 2) characterize the degrees of decomposition within the marsh’s C pools; and, 3) assess how these C pools and their degree of decomposition change within the marsh spatially and over the growing season.

3.2 Methodology

3.2.1 Site description

Our site is part of the Mer Bleue wetland complex, east of Ottawa, Ontario Canada (45.40 °N, 75.50 °W) which spans 2800 ha of ombrotrophic bogs, nutrient poor fens, and marshes (Fig. 3.1a). The study site is a cattail-dominated freshwater marsh residing within the southwestern tip of the complex (Bonneville et al., 2008).

The wetland complex formed in an abandoned river channel on the Ottawa clay plains and developed due to the poor drainage of the area (Geological Survey of Canada, 1987; Ministry of Agriculture and Food Ontario, 1987). Mer Bleue is a natural conservation area managed by the National Capital Commission; however, it has been subject to human disturbances such as roads, drains, and railways. Unsuccessful attempts were made, starting in the 1800s, to drain the wetland complex with the Lacroix and Savage Municipal Drains, which are in the northern finger and on the eastern side of the complex respectively (Fig. 3.1a) (National Capital Commission, 1994).

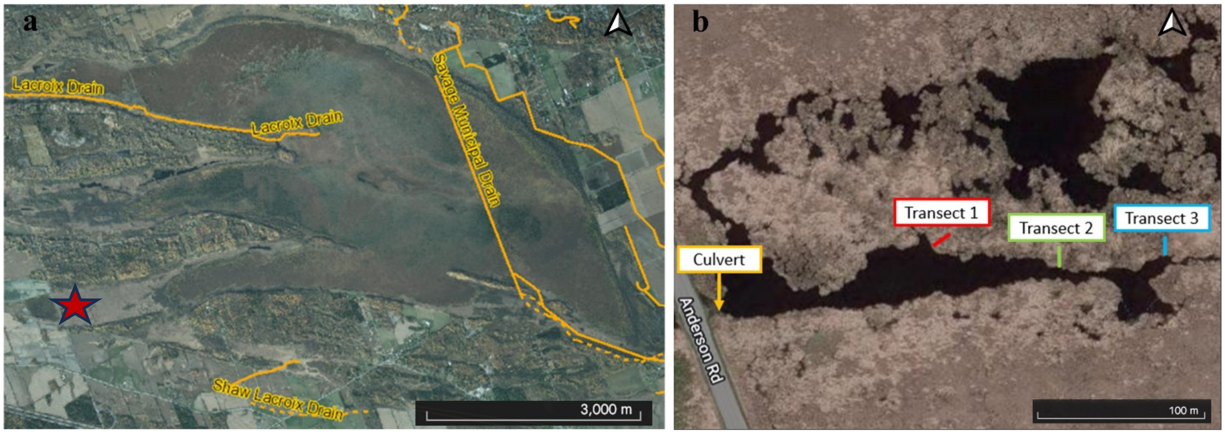

Figure 3.1 (a) Satellite image of the Mer Bleue wetland complex and location of the *Typha* dominated marsh on its southwestern tip (indicated by star). Yellow lines indicate constructed drains in the area. (b) Layout of study sampling transects in the marsh and the location of the culvert where marsh water outflow occurs. Sources: (a) Ontario Ministry of Agriculture, Food and Rural Affairs, 2023; (b) Google Maps, 2023.

The marsh is dominated by narrow-leafed cattail (*Typha angustifolia*) that grow in monostands. Upstream (on the eastern side), the marsh is connected to the peatlands of the larger Mer Bleue complex, while downstream (on the western side) it is bordered by Anderson Road. An open water channel runs through the marsh from east to west, leading to a single culvert that serves as the output for the marsh's water (Fig. 3.1b). The marsh's soil is sapric (characterised by black, well-decomposed organic material) underlain by mineral soil. The regional climate is temperate with a mean annual temperature of 6.5 °C and a mean annual precipitation of 929.8 mm (Environment and Climate Change Canada, 2023).

Sampling took place throughout the growing season of the *Typha*, starting on May 24th when *Typha* shoots began to emerge and ending on November 9th when *Typha* were senesced. Within the *Typha* stands, three transect locations were selected, and three boardwalks were constructed (perpendicular from the open water channel) using 2 x 6” wood planks to facilitate access to the sampling sites (Fig 3.1b). Along each transect, sampling points were located at 0.5 m, 1.0 m, 1.5 m, and 3.0 m away from the open water channel. The location and length of the transects allowed for the observation of changes in C pools both along the channel from upstream to downstream, and by relative proximity to the open water channel along each transect.

3.2.2 Aboveground and belowground biomass sampling

We harvested the aboveground biomass of *Typha* stalks (leaf, stem, and reproductive organ) on ten dates, starting on May 24th (when the *Typha* emerged) and ending November 9th, 2022 (*Typha* senescence). *Typha* were harvested on the southern side of the open water channel at distances of 30, 60, and 120 m from the channel, extending 10-20 m into the stands from Anderson Road. At each distance, 3-4 replicate *Typha* aboveground biomass samples were collected (n = 10). *Typha* density was recorded using a 0.25 m^2 quadrat.

We harvested belowground biomass before leaf-out on May 9th and after peak growth on August 20th 2022, at sampling points located 0.5, 1.0, 1.5, and 3.0 m from the open water channel. We cut approximately 30 × 30 cm sections of the *Typha* mat using a jab saw, placed them in marked opaque bags, and transported them to the laboratory for drying. Sampling was conducted along each of the three transects (Fig. 3.1b), which acted as replicates at each distance, resulting in a total of 24 samples.

3.2.3 Soil and water sampling

Soil within the *Typha* stands was sampled on May 9th 2022. We accessed the soil through the openings created by the belowground biomass sampling along the three transects at each distance. The transects were used as replicates for the soil samples. At each sampling point (n=12), we used a Dutch auger with a 5 × 20 cm thread to take three replicate cores at increasing depth, for a total of 36 soil samples. The complete soil core was ~60 cm in length and included the organic soil layer transitioning into the mineral soil layer. The organic layer was ~0-10 cm deep, and the mineral layer was at least four times that length (10- 60 cm deep). Each individual core was kept in a separate freezer bag and frozen at -20 °C until they could be analyzed for C and N.

Water was sampled nine times from June 8th to November 9th, 2022, within the *Typha* stands (at each of the four sampling points along the three transects), as well as at the culvert (at both its entrance and exit). We took three replicates from each of these sample points (n = 36 for samples taken within the *Typha* stands; n = 6 at the culvert). We collected water samples 30 cm below the water's surface using Nalgene bottles, accessing the water through openings created by harvesting belowground biomass in May. Upon retrieval from the field, we froze water samples at -20 °C until filtration and analysis for total dissolved organic C and total dissolved N (TDN) could be conducted. A significant rainfall event of 105.5 mm on August 7th and the morning of the 8th preceded water sampling in the marsh on August 8th, 2022.

While collecting the water samples, we simultaneously measured water temperature, depth, and flow. Water depth was measured at each sampling point, while temperature was recorded only at Transect 1 using a bimetallic-coil thermometer. Water flow was measured with a pygmy meter, but each attempt resulted in a flow measurement of 0.0 m s^{-1}, either due to obstruction from surrounding plant matter or water flow being below the stall speed of the flow meter.

3.2.4 Biomass dry weight

The aboveground biomass was air-dried and then separated into three components: leaf, stem, and reproductive organ; the dry mass of each component was then determined. The belowground biomass was first air-dried until most of the moisture evaporated, then oven-dried at 60 °C for 48 hours at which point the total mass of the dry belowground biomass was measured. Subsamples (~10 g) of the dried leaves, stems, reproductive organs, and belowground biomass were ground and stored in glass scintillation vials for later C and N analyses.

3.2.5 Soil processing

Soil samples were processed to quantify total soil organic C and total N, soil dissolved organic C and total dissolved N, and total soil $\delta^{13}C$ and $\delta^{15}N$ isotopes. The soil was thawed until soft enough to cut into three distinct layers: organic, transitional, and mineral. The organic layer was dark sapric peat, and the mineral layer was made of grey-blue clay, while the transitional layer was a mixture of organic and mineral materials and contained a thin layer of sand within the clay. The composition of the transitional layer is characteristic of the soil in the area, where the soils are underlain by marine clay covered with a thin layer of fluvial sand (Geological Survey of Canada, 1987).

The three distinct soil layers were homogenized separately, and two subsamples were extracted, each weighing 15.0 ±1 mg of wet soil. One subsample was placed in 40 mL falcon tubes and frozen at -20 °C for subsequent DOC and TDN analysis. The second subsample was measured wet into tins and oven-dried for gravimetric water content. The tins containing the organic and transitional layers were oven-dried at 50 °C due to their organic contents, while the mineral layer was oven-dried at 105 °C. Once dried, we ground the organic and transitional layers to <53 µm on a shaker using ball bearings for fourteen hours. The mineral soil was ground with a mortar and pestle. All ground soil samples were stored in glass scintillation vials until analyzed for total soil organic C, total N, and for δ^{13}C and δ^{15}N isotopes.

3.2.6 Soil and water DOC and TDN analyses

To extract soil DOC and TDN, the subsampled 15.0 ±1 g of wet soil stored at -20 °C was thawed at 4 °C, 40 mL of double-deionized water was added to each sample, and then samples were placed on a shaker for 20 m before centrifuging for 15 m at 8,500 rpm. From each sample, we vacuum-filtered 50 mL of this solution (which included soil water) using glass fibre filters with 47 mm ⌀ and a 37 µm pore size to remove particulate matter. The water samples were thawed at 4 °C and briefly shaken by hand before filtering 50 mL using the same filtration system as the soil samples. Both soil and water filtrates were stored at -20 °C until the DOC and TDN analyses.

We analyzed the DOC and TDN of soil and water filtrates on a TOC/N analyzer (TOC-L Series, Shimadzu Corp, Kyoto, Japan). For soil, 10 mL of filtrate was diluted with 20 mL of double-deionized water and acidified with a drop of 1M hydrochloric acid before running it on the TOC/N analyzer. Water DOC and TDN are reported as mg C L^{-1} and mg N L^{-1} and soil DOC and TDN were converted to g C kg^{-1} dry soil and g N kg^{-1} dry soil

3.2.7 Soil and plant biomass dry total C and N and stable isotope analysis

Once ground, we treated the above and belowground biomass and soil identically for total C and N analysis. The analyzed sample mass depended on the organic content of the C pool: 7.0 ±1 mg for plant biomass and the soil organic layer, 15.0 ±1 mg for the soil transitional layer, and 20.0 ±1 mg for the soil mineral layer. We determined total soil and plant C and N on a flash combustion elemental analyzer (Costech EA ECS 4010, Valencia Ca, USA), reported as g C kg^{-1} and g N kg^{-1} dry biomass or dry soil.

We conducted $\delta^{13}C$ and $\delta^{15}N$ analysis of plant biomass and soil on an Isolink Flash Elemental Analyzer paired with a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany). Not all plant biomass samples that were collected were used to determine total plant ^{13}C and ^{15}N. Instead, we analyzed three analytical replicates (5.50 ± 0.3 mg), homogenized from the *Typha* aboveground biomass harvested on November 9th 2022, across all locations. At this date, the *Typha* were senesced and at the end of their live C cycle before decomposing and transitioning into a new C pool. We created analytical replicates by first determining the average percent mass of each plant part: 78% stem, 13% leaf, and 9% reproductive organ. Based on these percentages, the mass of each part per plant was determined and homogenized as described above. For the belowground biomass, we analyzed three analytical replicates (1.50 ± 0.01 mg) of the belowground biomass harvested on August 20th 2022, which were homogenized across all locations for ^{13}C and ^{15}N.

We analyzed the three soil layers (organic, transitional, and mineral) for ^{13}C and ^{15}N at Transects 1 and 3, with sample masses as follows: organic to 2.00 ± 0.01 mg, transitional to 8.00 ± 0.4 mg, and mineral to 40.0 ± 2.00 mg. Transects and sample points along transects were treated as replicates. The samples at each location were kept separate resulting in nine organic, seven transitional, and eight mineral replicates for each soil layer.

3.2.8 Data analysis

To ensure data integrity, we examined the instrument's data output for outliers through percent differences in analytical replicates; samples with analytical replicates with percent differences over 5% were rerun. We conducted statistical analysis on R version 4.2.3., where significance was established at $\alpha = 0.05$. We employed one-way ANOVA paired with Tukey's range test to assess differences between C and N concentrations, C:N, $\delta^{13}C$, and $\delta^{15}N$. Treatments for these tests were the different organic pools, the sample location (transect and sampling point along transects) and sampling dates. We also distinguished water samples' locations within *Typha* stands and at the culvert (water output of the marsh). We took three replicates at each sample location. When we observed no differences in C, N, and C:N values of the organic pools between locations (transects and sampling points), we treated the sampling points as replicates. Sampling dates were used to compare how C and N concentrations, and C:N values changed throughout the growing season for water, and above and belowground biomass samples. We applied linear and logarithmic regression models to investigate temporal and spatial trends in C and N concentrations, C:N, $\delta^{13}C$, and $\delta^{15}N$, as well as relationships between C and N concentrations, and $\delta^{13}C$, and $\delta^{15}N$. These relationships were explored visually through line plots. We used a repeated measure ANOVA to compare the DOC and C:N of the water samples taken within the *Typha* stands and at the culvert at each of the sampling dates throughout the growing season.

3.3 Results

3.3.1 Total carbon in pools

Belowground biomass, water within *Typha* stands, soil dissolved organic matter (DOM) and soil organic matter samples taken along the transects did not differ in C concentrations or C:N by distance from the open water channel. We therefore treated these points as replicates. The highest mean carbon concentration among solid-state pools was found in the aboveground dry biomass (43.0% C), whereas the lowest among dissolved pools was DOC at the culvert (33.9 mg C L^{-1}) (Fig. 3.2). Belowground dry biomass had the second highest C concentrations, averaging 417 g C kg^{-1}. The high variability in aboveground biomass C reflects the change in growth of the *Typha* over the season. Mean DOC in the *Typha* stand was 1.29 times higher than the culvert DOC ($p < 0.05$). However, soil DOC was similar among all soil layers. Total soil C was highest in the organic layer, 5.31 and 12.9 times greater than the transitional and mineral layers ($p < 0.05$). The transitional layer was also greater than the mineral layer by 2.42 times the amount (a difference of 39.0 g C kg^{-1}) ($p < 0.05$).

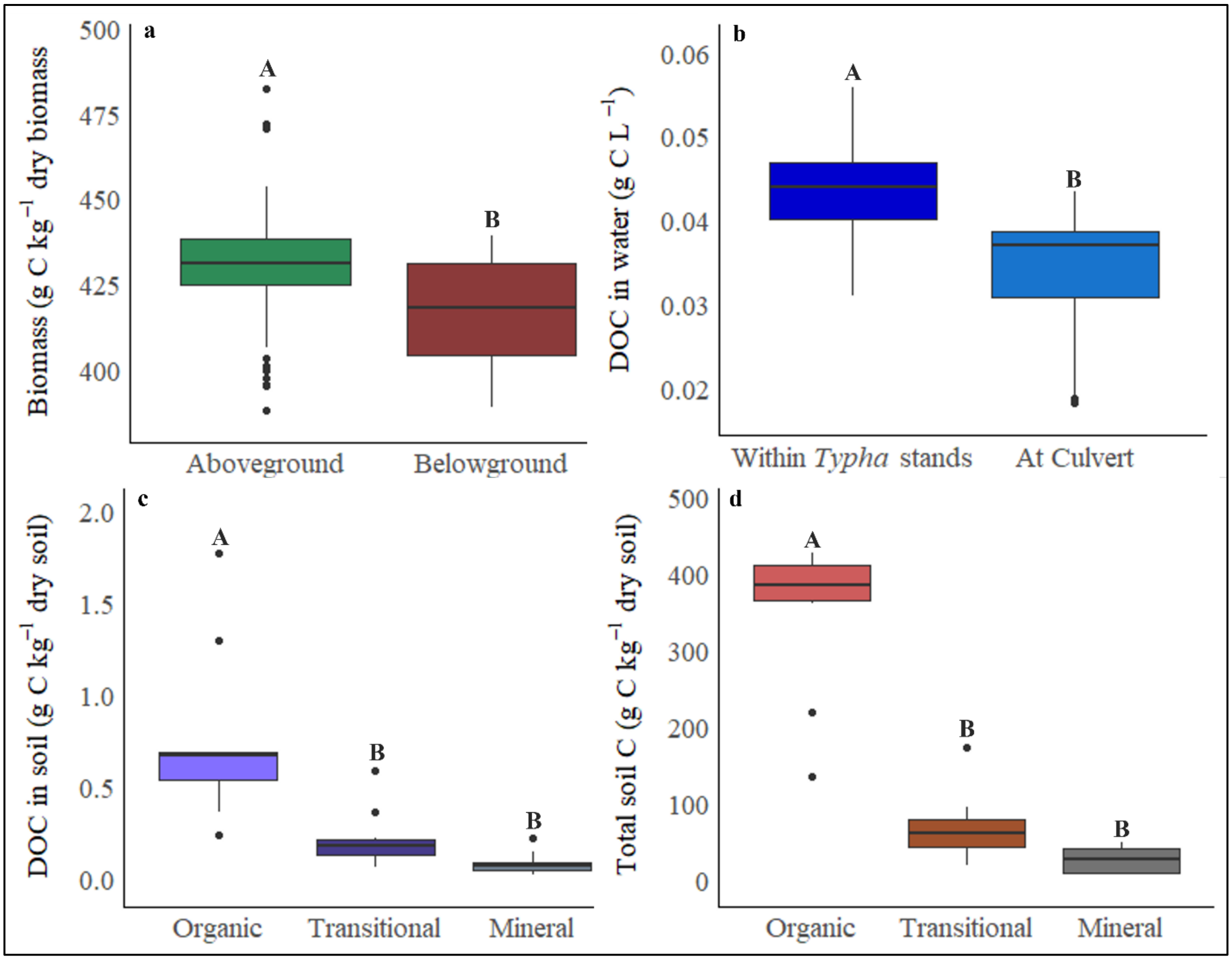

Figure 3.2 Carbon concentrations in the organic matter pools of the Mer Bleue *Typha*-dominated freshwater marsh during the 2022 growing season. (a) Aboveground dry biomass from June 8th, July 30th, August 8th, and November 9th (n = 10); belowground biomass of the *Typha* stands from May 24th and August 20th (n = 12) ; (b) Water dissolved organic carbon (DOC) from the outflow culvert (n = 6) and within *Typha* stands (n = 36) from nine sampling dates between June 8th and November 9th; (c) Soil DOC collected in May from the organic (n = 10), transitional (n = 11), and mineral (n = 11) layers of the soil profile; (d) Total soil C from the organic (n = 10), transitional (n = 11), and mineral (n = 11) layers of the soil profile from May soil samples. Uppercase letters indicate significant differences within each organic matter pool at α = 0.05.

The aboveground dry biomass C:N (mean 67.1) was the highest of all the other organic matter pools, while the DOM in the soil had the lowest (Fig. 3.3**).** The high C:N variability in the biomass and water DOM samples reflects seasonal variation across multiple sampling dates, whereas the lower variability in soil samples reflects only spatial variation from the May sampling date. The C:N of soil DOM and total soil were similar across layers and, even though they were not significantly different from each other, the mean C:N of soil DOM (ranging from 10.1-11.1) was around half of the mean C:N of the total soil (ranging from 22.7-18.1). The lowest C:N values were from soil DOM samples, including the water DOM C:N which exhibited these values towards the end of the season in November (see Fig. 3.6).

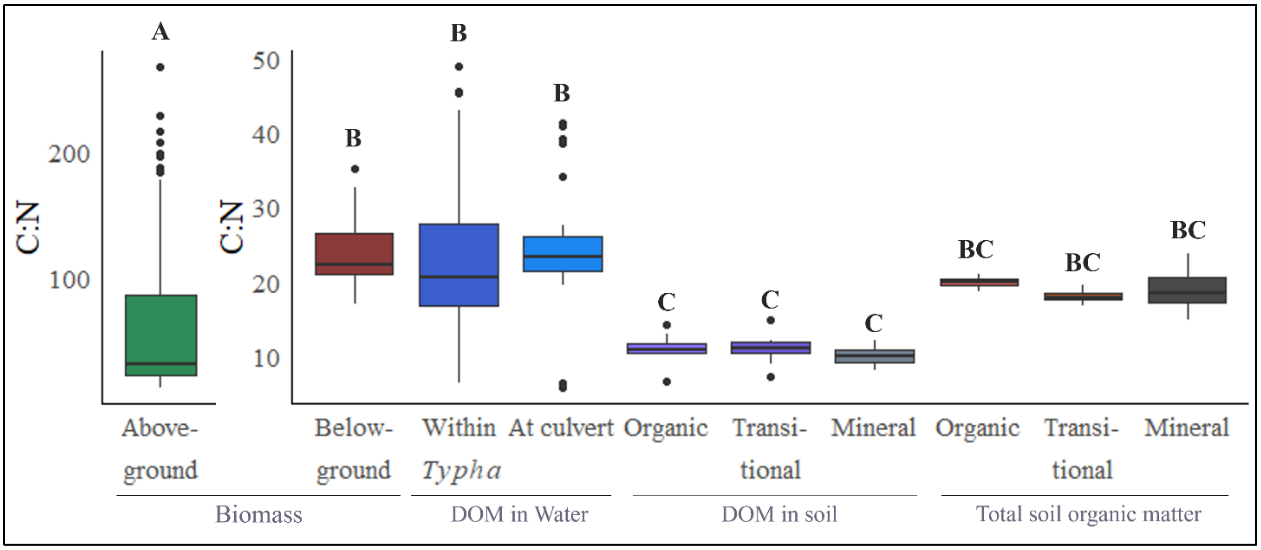

Figure 3.3 C:N of organic matter pools in the *Typha*-dominated freshwater marsh. Differences in C:N are quantified through Tukey's range test. Uppercase letters indicate significant differences between and within organic matter pools at α = 0.05. Additional information on the sampling of the pools can be found in Figure 3.2.

3.3.1.1 *Typha* biomass

3.3.1.1.1 Aboveground biomass

Typha aboveground biomass increased from emergence in May until reaching a peak at the reproductive stage in August (1747 g dry biomass m^{-2}) with an average of 68 plants m^{-2}. Between the reproductive stage and senescence, we observed no change in biomass (Fig. 3.4). After senescence, aboveground dry biomass decreased by half to 957 g m^{-2}. The majority of the *Typha*'s aboveground dry biomass was allocated to the stem, constituting 72% of the total mass.

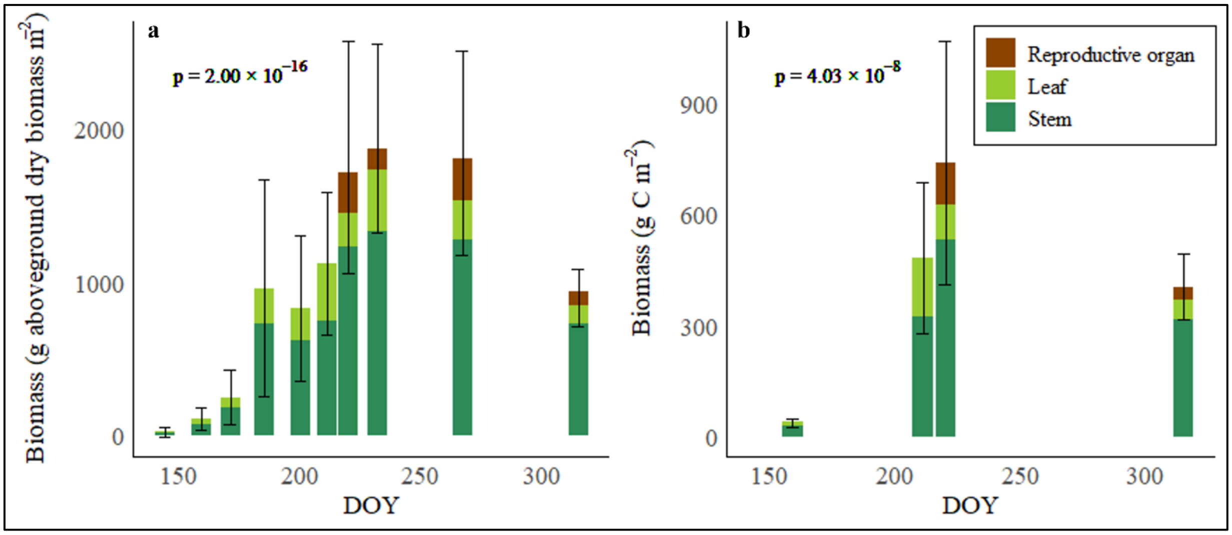

Figure 3.4 Aboveground biomass partitioning into different Typha components, including reproductive organ, leaf, and stem throughout the 2022 growing season from sampling on the southern side of the open water channel at distances of 30, 60, and 120 meters from the channel, extending 10-20 meters into the stands from Anderson Road. (a) Mean (n=10) Typha aboveground dry biomass per m^2 sampled on 10 dates between May 24th and November 9th (b) Mean Typha aboveground biomass C concentration per m^2 (n=10), for four sampling dates June 8th, July 30th, August 8th, and November 9th, representing the different Typha growth stages. Error bars and p value indicate the standard deviation of total mass between plants.

3.3.1.1.2 Belowground biomass

From May to August, 2022, we observed the mean belowground dry biomass C concentration decreased by 14 g C kg^{-1} (Fig. 3.5a). Similarly, C:N decreased between the two dates from 25.2 to 20.8 (Fig. 3.5b), while the mean C g m^2 increased non-significantly by 1830 g C m^2 (Fig. 3.5c). The overall mean g C m^2 of the belowground biomass (5168 g C m^2) was ~ 7 times higher than that of the aboveground biomass at its peak (Fig. 3.4b and Fig. 3.5c).

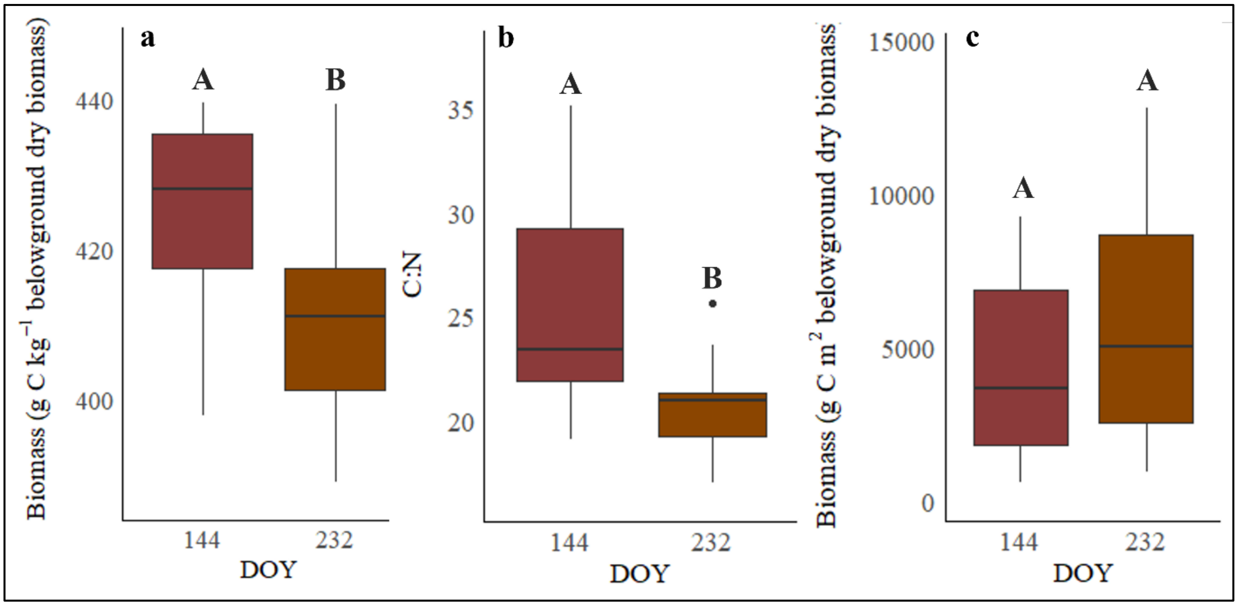

Figure 3.5 Belowground biomass for sampling dates at emergence (DOY 144, May 24th) and peak biomass (DOY 232, August 20th). (a) C concentration (n=12), (b) C:N (n = 12), and (c) C mass per m^2 of the belowground dry biomass (n=8). Differences are quantified through Tukey's range test. Uppercase letters indicate significant differences between the sampling dates at $\alpha = 0.05$.

3.3.1.2 Water DOC

DOC concentrations varied between transects. The middle transect (Transect 2) had a lower mean DOC (41.6 mg C L^{-1}) compared to the transects closest and furthest from the inflow (Transects 1 and 3; 44.6 and 44.9 mg C L^{-1}, respectively), indicating no discernible linear trend from upstream to downstream within *Typha* stands. However, mean DOC concentrations within *Typha* stands (43.7 mg C L^{-1}) exceeded those at the culvert (marsh output) (33.9 mg C L^{-1}).

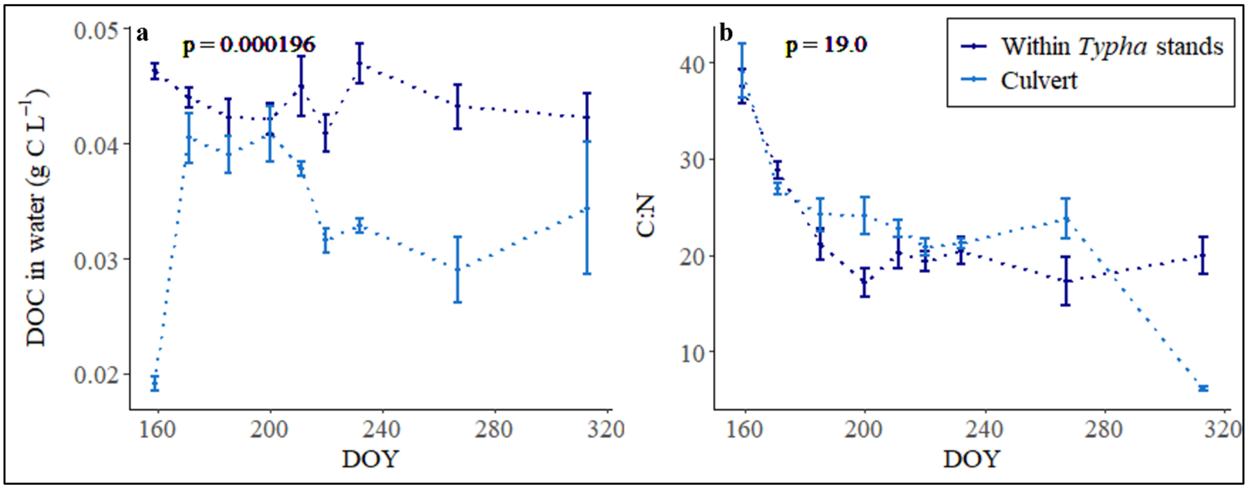

Figure 3.6 Marsh water sampled within Typha stands and at the culvert on June 8th, June 20th, July 4th, July 19th, July 30th, August 8th, August 20th, September 24th, and November 9th (DOY: 159, 171, 185, 200, 211, 220, 232, 267, and 313) 2022 for (a) DOC concentration, and, (b) Dissolved C:N. Differences in C and C:N are quantified through Tukey's range test. The p value is representative of a repeated measures ANOVA.

DOC concentrations within *Typha* stands exhibited seasonal variation (Fig. 3.6a), reaching a peak at 46.9 mg C L^{-1} on August 20th (DOY 232) and a low of 40.9 mg C L^{-1} on August 8th (DOY 220). The mean DOC concentrations decreased from late spring to early summer (DOY: 159-200; June 8th to July 19th) at 0.102 mg C L^{-1} per day ($p < 0.0001$; $R^2 = 0.164$), and again late summer to fall (DOY: 232-313; August 20th to November 9th) at 0.0572 mg C L^{-1} per day ($p < 0.0001$; $R^2 = 0.102$). DOC increased mid-summer at a rate of 0.144 mg C L^{-1} per day ($p < 0.0001$; $R^2 = 0.113$), when excluding the August 8th (DOY 220) sampling date that was associated with a major rainfall event.

We found that the water at the culvert exhibited the lowest DOC concentration of the season on June 8th (DOY: 159) at 19.1 mg C L^{-1}, when the hydrophytes in the open water channel had not yet emerged. The DOC concentrations at the culvert peaked earlier than those within the *Typha* stands on July 4th (DOY: 185) at 40.8 mg C L^{-1}. DOC concentrations in the water column within the *Typha* stands were consistently higher than those at the culvert throughout the growing season, especially on June 8th, August 20th, September 24th, and November 9th (DOY: 159, 232, 267, and 313; Table S1).

The dissolved C:N of the water within *Typha* stands decreased logarithmically throughout the growing season ($p < 0.0001$; $R^2 = 0.242$) (Fig. 3.6b), and was not significantly different from the values found at the culvert with the exception of the samples from November 9th (DOY: 313) where the culvert's dissolved C:N reached a low of 6.12 (Table S2). Excluding the sampling dates of June 20th and November 9th (DOY: 171 and 313), the dissolved C:N of the water at culvert was higher than that of the water found within the water found within the *Typha* stands.

3.3.1.3 Total soil C and soil DOC

Total soil C decreased with depth (but not significantly), but the C:N did not (Fig. 3.2d and Fig. 3.3). The surface organic soil layer had the highest mean C concentration at 352 g C kg^{-1} dry soil, and the bottom mineral soil layer had the lowest at 27.3 g C kg^{-1} dry soil. Soil DOC and dissolved C:N also exhibited a non-significant decreasing trend with depth in the soil profile (Fig. 3.2c and Fig. 3.3).

3.3.2 Biomass and soil C and N isotopic composition

The mineral soil layer exhibited the highest mean $\delta^{13}C$ of -25.5 ‰ and differed from all the other C pools except that of the transitional soil layer (Fig. 3.7a). The remaining C pools exhibited similar $\delta^{13}C$ to each other, where the belowground dry biomass had the lowest mean $\delta^{13}C$ at -28.7 ‰. The soil layers had higher mean $\delta^{13}C$ than the biomass and increased with depth, although not significantly. The aboveground dry biomass had a higher mean $\delta^{13}C$ than the belowground dry biomass.

In terms of $\delta^{15}N$, only the aboveground dry biomass differed from the other pools, having the highest mean value at 3.00 ‰ (Fig. 3.7b). Conversely, the belowground dry biomass had the lowest mean $\delta^{15}N$ at 0.332 ‰.

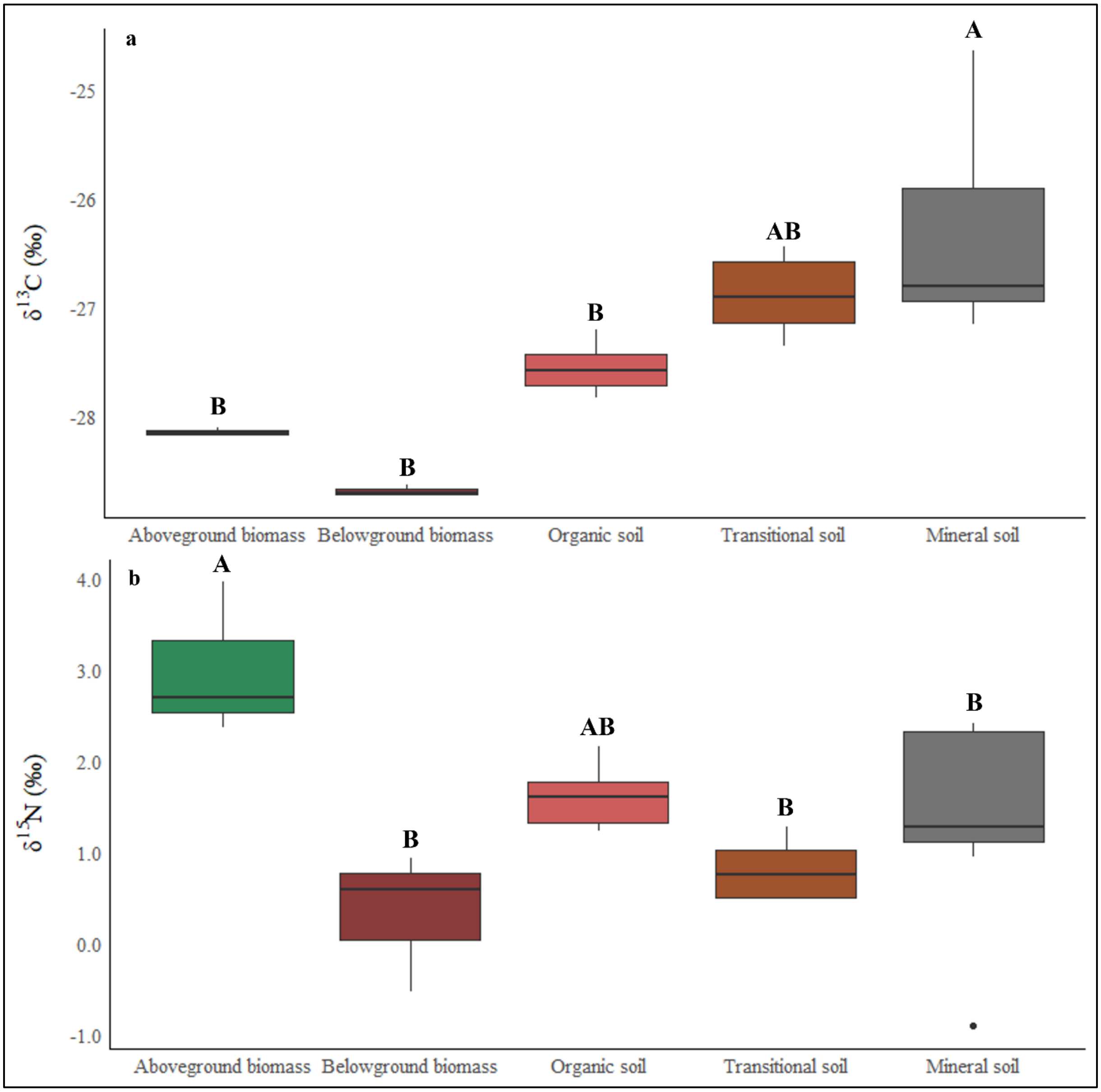

Figure 3.7 Isotope variation of (a) $\delta^{13}C$ in C pools, and (b) $\delta^{15}N$ in N pools of a Typha-dominated freshwater marsh during the 2022 growing season. Differences in $\delta^{13}C$ and $\delta^{15}N$ are quantified through Tukey's range test. Aboveground dry biomass samples are three replicates from November 30th. Belowground dry biomass of the Typha samples are three replicates from August 20th. Total soil C samples from the organic, transitional, and mineral layers of the soil profile were sampled in May. Uppercase letters indicate significant differences between and within each organic matter pool at $\alpha = 0.05$.

There were no differences in $\delta^{13}C$ or $\delta^{15}N$ in any of the soil layers by distance from open water channel and so these points were treated as replicates. Within the soil layers, $\delta^{13}C$ shared a negative logarithmic relationship with the C concentration (Fig. 3.8), while $\delta^{15}N$ shared no relation with the N concentration (data not shown).

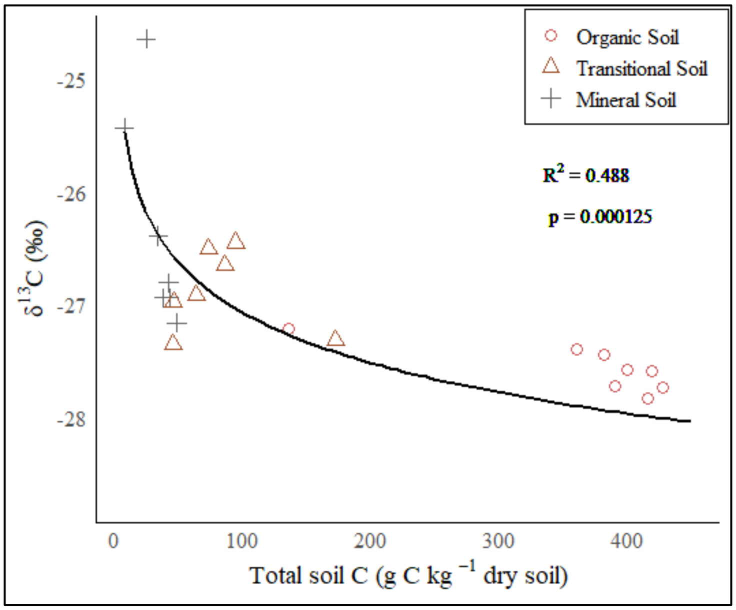

Figure 3.8 $\delta^{13}C$ and C concentration in the marsh soil layers as indicated by the symbols

3.4 Discussion

Freshwater marshes, such as the *Typha*-dominated system studied here, act as significant C sinks. Despite their role in global C storage, they are often overlooked compared to peatlands, primarily due to their relatively smaller peat deposits. Most research focuses on the accumulation of SOC in peat layers, often neglecting C stored in the mineral soil layers, which are characteristic of freshwater marshes (Loder & Finkelstein, 2020). To address this gap, our study quantified and characterized the different pools of C in a freshwater marsh and examined their spatial and temporal dynamics. Our results show that a substantial portion of the C in this ecosystem is stored in the belowground biomass of *Typha*. Despite the pronounced temporal dynamics, spatial variation in C concentrations across transects was minimal, but differed from the outflow, suggesting C is staying in place rather than moving downstream. Further, the *Typha* biomass drives much of the marsh's C storage, but we found that the mineral soil layer, may act as a secondary, more stable C reservoir.

3.4.1 Seasonal variation of C pools and potential origins of DOC

Throughout the growing season, aboveground biomass increased, peaking in late August (Fig. 3.4b), consistent with previous studies on the Mer Bleue marsh (Bonneville et al., 2008; Strachan et al., 2015). Strachan et al. (2015) proposed that the atmospheric C, gained from these high levels of primary productivity, is likely translocated into root mat production and peat deposits, which this study supports.

Typha's relatively high lignin content (~19.4% in aboveground biomass and ~17.8% in belowground, compared to <15% in most herbaceous plants), along with the marsh's anaerobic conditions, results in slow decomposition and the accumulation of the *Typha*'s biomass as peat in the root mat (Adingo et al., 2021; Li et al., 2019; Reddy & DeLaune, 2008; Sharma et al., 2020). However, because we did not measure oxygen levels in the marsh, we cannot quantify the exact level of anoxia. Nonetheless, during warmer months, senesced *Typha* biomass decomposes more rapidly due to increased microbial activity (Atkins et al., 2017), which is reflected in decreasing biomass C:N, as more C is lost during decomposition than N (Chapin et al., 2011; Reddy & DeLaune, 2008). We observed this trend where belowground biomass C:N decreased from 25.2 to 20.8 between May and August (see Fig. 3.5b, 3.5c). At the same time, root growth and thus new belowground biomass inputs also typically peak during warmer months, which could replace C lost during decomposition and mask changes in belowground C:N associated with decomposition. In our study, while we found that mean belowground biomass in the root mat increased by 1830 g C m^{-2} during the summer, it was non-significant and thus may not be rapidly replacing decomposed biomass with new inputs. Nonetheless, the root mat had ~7 times more g C m^{2} than the aboveground biomass at peak mass, likely because of its high density and accumulation from previous years (Fig. 3.4b, 3.5c; Sharma et al., 2008). This accumulated root biomass is often unaccounted for in peat wetland studies, despite it representing a significant C stock in the marsh.

Some *Typha* biomass C may be transported out of the system, when decomposed into DOC, either through lateral water movement or uptake and mineralization by microorganisms (Ågren et al., 2010). Freshwater marsh water DOC concentrations fluctuate seasonally, influenced by factors such as root exudates, upstream inputs, water velocity, and both abiotic and biotic decomposition (Bansal et al., 2019; Flanagan et al., 2006; Hensgens et al., 2020; Marx et al., 2017). By comparing temporal trends in DOC and the C:N of DOM within the *Typha* stands to the marsh's outflow (the culvert), we can infer whether DOC is accumulating in the marsh or being lost downstream, and assess whether DOC within the *Typha* stands originates from newer sources like root exudates or older C pools, such as the belowground biomass decomposition. If the DOC primarily originates from decomposing belowground biomass, it suggests that detritus root inputs may not be effectively retained in the long-term.

In the Mer Bleue marsh, DOC in the water column ranged from 40 to 80 mg C L^{-1}, consistent with other locations (Fig. 3.6a; e.g., Chu et al., 2015). DOC concentrations at the culvert were lower than those within the *Typha* stands and did not always share the same trend. Early in the growing season (June 8th) the culvert DOC was ~2.4 times lower than within the *Typha* stands (Fig. 3.6a). This difference suggests that downstream DOC movement may have been impeded by the *Typha* root mat remaining frozen until the last week of May. The frozen period would likely reduce the time during which DOC could be influenced by lateral water movement, microbial activity, and *Typha* root exudate before the June 8th sampling date. After July 19th, culvert DOC decreased dramatically, while DOC within the *Typha* stands and the *Typha* aboveground biomass both peaked on August 20th. Moreover, some culvert DOC could originate *in situ* rather than upstream, for instance, from the algae we observed at the culvert after June 8th. These data suggest that some DOC produced in the *Typha*, either as fresh inputs or during decomposition, remains within the marsh, at least in the short term. More frequent sampling and gross DOC production rates are needed to confirm these patterns, while DOC chemistry could further aid in tracking the DOC trajectory from upstream to downstream. For example, if culvert DOC is primarily from decomposition rather than fresh *Typha* inputs, we would expect lower abundances of lignin and aromatic compounds in DOC (Neff et al., 2006). Similarly, a decrease in the dissolved C:N in the water column over time and from upstream to downstream, can indicate greater contributions from decomposition, providing insight into the DOC's pathway through the system and its origin.

The dissolved C:N at both the culvert and the *Typha* stands were highest at the start of the growing season, coinciding with new *Typha* shoot growth (Fig. 3.5b). After aboveground biomass was established, dissolved C:N within the *Typha* stands remained relatively consistent, indicating minimal contributions from decomposition and relatively more or continued input from fresh *Typha*. Alternatively, the N in the *Typha* stands' water column may be lost to plant and microbial uptake at a higher rate than DOC mineralization, or it may be removed through sedimentation and denitrification (Saunders & Kalff, 2000), masking what would have otherwise shown a decreasing C:N. However, DOC concentrations within the *Typha* stands did not decrease substantially throughout the season and peaked during the period of maximum aboveground biomass, further suggesting contributions from increased root exudates. DOC within the *Typha* stands can decompose on its way to the marsh's output (the culvert), which could lower dissolved C:N given a slow enough transit time (Marín-Spiotta et al., 2014). However, this is not what we observed. Instead, the dissolved C:N was higher at the culvert throughout the growing season, except for June 20th and November 9th when large amounts of detritus accumulated at the culvert. This relatively higher dissolved C:N at the outflow further indicates that the DOC leaving the system consists of newer inputs. If DOC losses from transport are primarily from new *Typha* inputs and not from older decomposed biomass, this DOC loss is a small fraction relative to total new *Typha* C production and would contribute to Mer Bleue being a C sink. The DOC retained within the *Typha* stands, may also decompose at a slower rate than if transported downstream. Though some of this retained DOC is expected to contribute to the marsh's CH_4 and CO_2 emissions, it can also be sorbed onto mineral surfaces, possibly increasing its residence time.

3.4.2 C movement and stabilization in the marsh soil

C accumulates in marsh soils through the deposition of DOC and POC from both local biomass and upstream sources. This means that not only is the C produced *in situ* retained, but external sources are also accumulating in the soil. To assess the marsh's potential as a sink for this labile C, we examined the distribution of SOC and DOC across different soil layers, along with organic matter C:N and isotopic C and N signatures, providing insight into C retention within the ecosystem.

In the soil layers of the Mer Bleue marsh, we observed the expected distribution of soil C, where the organic layer had the highest C concentrations, and the mineral layer had the lowest. C concentrations within the organic layer were typical for freshwater marshes, ranging between 305–451 g C kg^{-1} dry soil (Lasota & Błońska, 2021). The mineral layer's C concentration, however, was nearly three times higher than the upper estimates previously reported (Wang et al., 2011; Zhang et al., 2008), likely due to the underlying marine clay in the Mer Bleue marsh.

Despite the mineral layer holding less C per gram of soil compared to the organic soil layer, it has a higher density and greater depth, meaning it may still provide a significant C sink. At Mer Bleue, the mineral layer is at least four times the depth of the organic layer (although we do not know the soil composition below 60 cm). We can use the mean percent C of the peat and mineral layers (35.2% and 2.72%) and their depths (0.1 m and 0.5 m) from our findings at Mer Bleue to estimate the C per area by depth in the marsh. If we assume a bulk density of the peat and mineral layers to be 0.2 Mg m^{-3} and 1 Mg m^{-3} (Mobilian & Craft, 2022; Tadiello et al., 2022), then the total C stock by depth in the mineral layer is estimated to be 136 Mg C ha^{-1}, and only 70.4 Mg C ha^{-1} in the peat layer. Based on these rough estimates, the mineral layer may be a significantly larger stock of marsh C compared to the smaller peat layer. This C sink potential in mineral soils is often overlooked, as most assessments only account for the organic soil layer of the marsh. Including the deeper C stored in the mineral soil may significantly increase the total C stocks per unit area, with the mineral layers potentially offering protection for long-term C storage. It is within the mineral soil layer that DOC and some decomposed POC sorbs onto mineral surfaces, protecting it from further microbial decomposition and mineralization (Emde et al., 2022; Mirabito & Chambers, 2023). This mineral protection may prove crucial with future anthropogenic disturbances where water DOC, root biomass, and peat layers may rapidly mineralize following drainage, but C in the mineral layer may remain protected and relatively persistent despite a change in the environment. The persistence of C in the marsh, however, is more pronounced when the ecosystem remains intact, as demonstrated by the gradual transformation of organic matter from live *Typha* biomass to the mineral layer.

We can interpret the movement and possible retention of C in the soil layers by considering the C:N and the $\delta^{13}C$ and $\delta^{15}N$ of the organic, transitional, and mineral layers. Typically, C:N decreases with increasing levels of decomposition, which is seen in well-drained mineral soils and some peatlands (Chapin et al., 2011; Hornibrook et al., 2000; Mirabito & Chambers, 2023; Reddy & DeLaune, 2008). Within the Mer Bleue marsh soil profile, the C:N was low (18.1-22.7) but showed no discernable trend and remained relatively consistent across the different soil layers. Others have also previously reported a lack of a consistent C:N decrease with soil depth and, in some cases, even an increasing C:N (Hornibrook et al., 2000; Liu et al., 2023).

In our study site, the C:N in the soil, regardless of the layer, is lower than both the belowground biomass and the DOM in the water column, indicating the decomposition of added new inputs and the older roots, before it accumulates in the soil. While we might expect the C:N to decrease from the peat to the mineral layer if decomposition continues in the soil, at this depth, the marsh is more likely anaerobic compared to the water column and the root zone. Thus, we suspect that once the organic matter is deposited (either as dissolved or particulate matter) in the shallow peat layer, the transport to the mineral layer occurs without substantial decomposition. However, notably, the soil dissolved C:N is only 10, half as much as the bulk soil and DOM within the *Typha* stands, which could also suggest that some decomposition may be occurring at this depth but under these anaerobic conditions, C-rich compounds such as phenolics, are preferentially interacting with mineral surfaces (Huang et al., 2020).

If the mineral layer is indeed accumulating more decomposed organic matter and the lack of a change in the C:N is rather due to differences between C and N retention, we would expect to still observe δ^{13}C enrichment with depth. Enrichment in δ^{13}C and sometimes δ^{15}N is often used as a proxy for increasing microbial processing and decomposition and typically increases with soil depth (Hobbie & Högberg, 2012; Krüger et al., 2023). We observed this depth-dependent pattern, where the organic layer (-27.5 ‰) was less enriched compared to the mineral layer (-25.5 ‰), consistent with the literature (Hornibrook et al., 2000; Minick et al., 2019). However, the change was small where the organic layer was only +0.7‰ more enriched and the mineral layer +2.7‰ more enriched than the senesced aboveground biomass. In addition to the consistent C:N between soil layers, these minimal changes in δ^{13}C signatures in relation to SOC concentrations (Fig. 3.7) are common in wetland soils, highlighting that while decomposition is limited at depth, it is not entirely halted (Minick et al., 2019), contributing to a low C turnover rate.

Typically, δ^{15}N increases with soil depth due to decomposition favoring the retention of heavier isotopes, a pattern observed in both peatlands and non-saturated mineral soils. However, similar to the C:N trend in the soil profile, δ^{15}N did not follow a linear trend with depth. Instead, it first decreased from the organic layer to the transitional layer, from 1.56 ‰ to 0.79 ‰, before increasing to 1.20 ‰ in the mineral layer. While δ^{15}N generally increases with the degree of decomposition in peatlands and non-saturated mineral soils, this pattern aligns more closely to other freshwater mineral wetlands (Choi et al., 2007; Delwiche & Steyn, 1970; Elliott & Brush, 2006). This trend stresses the complexity of the N pathways through the marsh's ecosystem that can often introduce other sources of δ^{15}N fractionation unrelated to decomposition. For instance, ion exchange can lead to δ^{15}N depletion, which may occur under the reducing conditions of the soil (Hubner, 1986). Further, the $\delta^{15}N$ signature depends highly on the form of N present and which part of the N cycling is dominating since N cycling includes several (often co-occurring steps) that can be either depleting or enriching (Högberg, 1997; Kendall, 1998; R. Wang et al., 2021).

Overall, these findings demonstrate that marsh soils, particularly the mineral layer, represent an important and often underappreciated C sink. While the organic layer stores a significant portion of marsh C, the deeper mineral layer, particularly with its greater depth, might be a more stable and long-term C reservoir. A more detailed characterization of the soil organic matter chemistry and measuring the C concentrations in the deeper mineral layers beyond what we measured (>60 cm), would help verify this. These insights underline the importance of including both organic and mineral layers in C stock assessments for more accurate estimations of marsh C storage potential.

3.5 Conclusions

Through this study we were able to quantify and explore the various C pools and dynamics of a freshwater temperate marsh, throughout a growing season. The *Typha*'s above and belowground biomass were the largest C stores of the system, with the belowground biomass equating to seven times the aboveground biomass. Although the organic matter in the belowground biomass became more decomposed as the growing season progressed, its overall mass increased. Both the organic and mineral layers of the marsh's soil were significant C pools, with the mineral layer's C concentration exceeding that observed in previous studies. While the organic layer had higher C concentrations, the greater density and depth of the mineral soil suggest it may represent a larger overall C pool. Proper bulk density measurements are needed to confirm this. The isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) and C:N ratios in the soil layers indicate limited decomposition at depth, especially in the mineral layer, which could aid in stabilizing C and enhancing retention. In particular, the mineral layer's depth and density, combined with its physiochemical properties from its high clay percentage, may provide long-term stability for the stored C, despite its lower concentration per gram of soil. Still, soil DOM C:N suggests continual decomposition at depth with lower C:N values compared to SOC. We also found evidence of DOC retention within the marsh, particularly in the *Typha* stands, indicating minimal downstream transport of older C from belowground biomass. Future studies could further investigate these findings through DOC flux measurements and the identification of C species from upstream to downstream. Together, these findings underscore the importance of considering both organic and mineral soil layers and the above and belowground biomass in marsh C stock assessments, as well as the role of seasonal and spatial dynamics in understanding marsh C retention.

3.6 References

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4. General Discussion

Climate change, driven by anthropogenic carbon (C) emissions, poses an escalating threat to our environment and society, making C sink ecosystems increasingly critical for mitigation (Loder & Finkelstein, 2020; Rydin et al., 2013; Villa & Bernal, 2018). Understanding the dynamics of C sinks—whether it is the amount these ecosystems store, sequester, or release—has become more important than ever. Wetlands are well-recognized as significant C sinks, but freshwater marshes remain understudied despite having many characteristics that suggest high C storage potential (Loder & Finkelstein, 2020; Mitsch et al., 2013; Rydin et al., 2013; Villa & Bernal, 2018). At the same time, they are at high risk of loss due to anthropogenic land-use changes (Byun et al., 2018).

Previous C gas flux research on the Mer Bleue marsh demonstrated its role as an overall C sink (Bonneville et al., 2008; Strachan et al., 2015), but quantifying the individual C stock, especially in the soil, remained necessary. The degree of vulnerability of C loss from an ecosystem due to disturbances, for instance climate or land use change, depends on where the C is stored within the ecosystem. Thus, by determining how C is distributed in Mer Bleue, we will have a better understanding of its longer-term storage potential. My thesis addresses this gap in the literature by investigating how C is stored and cycled within a *Typha*-dominated freshwater marsh in eastern Ontario. Through C analyses of field samples from the Mer Bleue marsh, I quantified the C concentrations in the organic matter pools of the biomass, water, and soil. These analyses also enabled me to assess the degree of decomposition by utilizing C:N alongside $\delta^{13}C$ and $\delta^{15}N$ analyses of the biomass and soil samples, providing insight into C movement and decomposition within these organic matter pools. By conducting field sampling throughout the

2022 growing season and across spatial gradients, I identified both temporal and spatial trends in C content and decomposition in the marsh’s organic matter pools.

Several findings from this study aligned with previous research on C stores, while others presented unexpected findings, opening new avenues for exploration in this field. For instance, while the expected C concentrations were observed in aboveground biomass, water, and soil dissolved organic C (DOC), as well as the organic soil layer, the mineral soil layer’s C concentrations exceeded those previously reported in the literature by threefold (Wang et al., 2011; Zhang et al., 2008). This discrepancy likely reflects the high clay content of the Mer Bleue marsh’s mineral soil, which enhances C retention (Bi et al., 2023). These findings underscore the importance of not treating marsh soils as uniform entities and reinforce the significance of understanding the underlying soil composition for accurate C storage estimations. Furthermore, when calculating the C of the different soil layers of the Mer Bleue marsh taking into account the depth of each layer, the mineral layer was estimated to have almost twice the C storage than the organic layer. This point echoes a concern in the lack of attention deeper soil C receives in soil science (Gross & Harrison, 2019).

Another key contribution of my study was the decision to measure both live and dead components of belowground biomass, an approach rarely adopted in similar research. Many studies on belowground biomass focus exclusively on live rhizomes and roots of *Typha*. However, the floating root mats of *Typha* also accumulate decomposing leaf and root litter from previous growing seasons, forming a peaty layer. Peat studies often overlook this peat accumulation within root mats, focusing instead only on the peat soil cores. By accounting for both live and dead components, my results revealed that belowground biomass serves as a substantial and previously underexplored C reservoir. Remarkably, C stored in the Mer Bleue belowground biomass was seven times higher per square meter than in aboveground biomass, with the additional benefit of acting as a longer-term C store.

Study limitations and future research

While this study provides valuable insights into C movement and storage in a freshwater marsh, certain methodological limitations warrant attention and raised additional questions. For instance, the water DOC analysis revealed higher DOC concentrations within the *Typha* stands compared to the marsh's output, suggesting reduced downstream DOC transport (likely due to low water velocity) and potential DOC retention within the system. This conclusion was based on comparisons of upstream and downstream DOC concentrations. Unfortunately, the low water velocity in the marsh fell below the stall speed of the analog velocimeter used, preventing accurate velocity measurements and, consequently, the calculation of DOC flux to confirm retention. Future studies could address this limitation by employing a digital water velocimeter capable of measuring lower velocities, enabling more precise assessments of DOC transport and retention.

DOC concentrations appeared to follow a seasonal trend correlating with aboveground biomass, leading to the hypothesis that root exudates from the biomass may drive these trends. Establishing causation would require more frequent sampling and analysis of DOC chemical composition. Techniques such as SUVA analysis could differentiate between DOC derived from fresh *Typha* inputs and older organic matter, deepening insights into seasonal DOC dynamics. Additionally, incorporating parallel particulate organic matter measurements could clarify how organic matter decomposition contributes to water column C throughout the season and reveal upstream versus downstream transport dynamics. This could be accomplished by replacing the

47 mm diameter glass fiber filters, used to vacuum filter water in the DOC analysis, with the 22 mm filters, as recommended by the University of Maryland Center for Environmental Science (2024). These smaller filters are compatible with elemental analyzers and can support both DOC and POC analyses.

In section 3.4, the C by depth of the marsh's soil layers was estimated using bulk density values from the literature, which challenged prior marsh soil C storage estimates that focused solely on the organic layer's peat reserves (Byun et al., 2018). To enhance precision, *in situ* bulk density measurements of the soils would have been crucial along with C concentrations of the full soil profile, below what we measured. While measuring bulk density is straightforward in well-drained soils using core or clod methods (Grossman & Reinsch, 2002), it becomes challenging in water-saturated soils due to compaction from water loss during sampling (Hargis & Twilley, 1994; Reddy et al., 2013). Alternative strategies, such as Hargis and Twilley's (1994) piston and cutting-edge auger or a core extruder as suggested by Folse et al. (2018), could mitigate compaction issues. While these methods require additional planning, their application would yield more reliable C estimates and advance understanding of marsh C storage potential.

When examining trends in C decomposition throughout the soil profile, the C:N ratios of the marsh soil's organic matter and dissolved organic matter (DOM) did not decrease significantly with depth. This pattern, commonly reported in the literature for waterlogged soils, contrasts with well-drained soils, where C:N typically decreases with depth. The underlying reasons for this phenomenon remain unclear (Hornibrook et al., 2000; Liu et al., 2023). These findings challenge the expectation that C:N decreases with increasing degrees of decomposition (Chapin et al., 2011; Hornibrook et al., 2000; Mirabito & Chambers, 2023; Reddy & DeLaune, 2008). Similarly, $\delta^{15}N$ also did not decrease with depth, which deviates from expectations that it should decline as decomposition and depth increase (Hobbie & Högberg, 2012; Krüger et al., 2023). While this lack of a trend is not unprecedented, it remains relatively underexplored in the literature (Hobbie & Ouimette, 2009; Kramer et al., 2017). Despite these anomalies, the lower C:N ratio of soil DOM compared to soil organic matter, along with the observed decrease in $\delta^{13}C$ with depth, does suggest some degree of decomposition within the soil profile. The combination of these unexpected patterns in C:N and $\delta^{15}N$ warrants further investigation, as it may provide valuable insights into decomposition processes and nitrogen dynamics in wetland soils. Future studies could test whether C in marsh soils is inherently more resistant to decomposition using controlled laboratory experiments.

Broader implications

Accurate estimates of marsh C storage inform us of the potential C loss that could result from their degradation, highlighting the extent of restoration efforts needed if these ecosystems are destroyed. This involves not only quantifying soil C in both the organic and mineral layers but also considering the peat within the belowground biomass of *Typha*. More comprehensive estimates of C stocks underscore the importance of preserving marshes, particularly given the persistent threat of land use change. Although wetlands have historically been undervalued and cleared for human development (Byun et al., 2018), this trend persists today. For instance, Northvolt, a Swedish company, plans to develop a battery plant on 138,162 m^2 of wetland in Saint-Basile-le-Grand, Quebec (*Centre Québécois du Droit de l'Environnement v. Procureur Général du Québec*, 2024), risking substantial C losses. A part of Northvolt's agreement with the Quebec government includes a commitment to restore an equivalent area of wetland to offset this impact, understanding the C value lost in wetlands is crucial. Restoration and conservation of wetland ecosystems are essential for mitigating rising atmospheric C levels.

Although my research contributes to advancing our understanding of marsh ecosystems and supports the role of freshwater marshes as significant C stores, additional studies are needed. Marsh soil composition and local conditions can influence C retention, complicating broader generalizations for models and larger-scale C storage estimates.

5. General Conclusions

This thesis underscores the critical role of freshwater marshes as carbon (C) sinks, particularly through their ability to store and cycle significant amounts of C within biomass, soil, and water. By focusing on the *Typha*-dominated freshwater marsh within the Mer Bleue wetland complex, this research adds to the growing body of knowledge on marsh C dynamics, a topic that remains underexplored compared to other wetland systems like peatlands.

A key outcome of this study was identifying the belowground biomass as the largest C pool in the marsh, with C stocks seven times greater than those of aboveground biomass. This finding highlights the need to consider both live and dead components of belowground biomass when evaluating marsh C stores, as traditional approaches often focus exclusively on live biomass. Additionally, soil C stocks proved substantial, with the mineral soil layer contributing significantly due to its high clay content, which enhances C retention through physiochemical stabilization. Remarkably, the mineral layer was found to store nearly twice as much C per area as the organic layer, emphasizing the importance of including deeper soil layers in marsh C assessments, as these are often overlooked in favor of surface organic soils.

Water column DOC concentrations revealed potential effective C retention within the marsh, with higher DOC concentrations observed within *Typha* stands compared to downstream outflow. These patterns suggest that the marsh functions as a DOC sink, reducing potential C losses to downstream systems.

Isotopic analyses and C:N ratios offered further insight into marsh C dynamics. Contrary to expectations, the C:N of soil organic matter and dissolved organic matter (DOM) did not decrease significantly with depth, and $\delta^{15}N$ showed no consistent pattern of enrichment. These anomalies, while not unprecedented in the literature, raise important questions about the unique decomposition and nitrogen dynamics in waterlogged soils. The observed $\delta^{13}C$ depletion with depth and the lower C:N of soil DOM compared to bulk organic matter suggest some degree of decomposition, though the processes governing these patterns require further investigation.

Overall, this thesis' findings demonstrate that these ecosystems not only sequester substantial C but also exhibit complex biogeochemical processes that govern its storage and decomposition. Moreover, it underscores the importance of integrating organic and mineral soil layers, incorporating live and dead biomass. These findings provide a foundation for future research and emphasize the value of conserving freshwater marshes as essential components of global C sequestration strategies in the face of climate change and anthropogenic pressures.

6. References

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6. Supplemental materials

Table S1. ANOVA of Mer Bleue marsh water dissolved organic carbon (DOC) comparing samples taken within *Typha* stands (n = 36) compared to samples taken at the culvert (n = 6) during 9 sampling dates from June 8th to November 9th, 2022.

DOY	p value
159	<0.05
171	NS
185	NS
200	NS
211	NS
220	<0.05
232	<0.05
267	<0.05
313	NS

Table S2. ANOVA of Mer Bleue marsh water dissolved C:N comparing samples taken within *Typha* stands (n = 36) compared to samples taken at the culvert (n = 6) during 9 sampling dates from June 8th to November 9th, 2022.

DOY	p value
159	NS
171	NS
185	NS
200	NS
211	NS
220	NS
232	NS
267	NS
313	<0.05