Dissolved Organic Matter Sources from Soil Horizons with Varying Hydrology and Distance from Wetland Edge

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ABSTRACT

Understanding hydrologic controls on carbon accumulation and export within geographically isolated wetlands (GIW) has implications for the success of wetland restoration efforts intended to produce carbon sinks. However, little is known about how hydrologic connectivity along the aquatic-terrestrial interface in GIW catchments influences carbon dynamics, particularly regarding dissolved organic matter (DOM) transport and transformation. The organic matter (carbon) that accumulates in wetland soils may be released into water, generating DOM. DOM is mobile and reactive, making it influential to aquatic metabolism and water quality. To understand the role of different soil horizons as potential sources of DOM, extractable soil organic matter (ESOM) was measured in soil horizons collected from upland to wetland transects at four Delmarva Bay GIWs on the Delmarva Peninsula in the eastern United States. ESOM quantity and quality were analyzed to provide insights to organic matter sources and chemical characteristics. Findings demonstrated that ESOM in shallow organic horizons had increased aromaticity, higher molecular weight, and plant-like signatures. ESOM from deeper, mineral horizons had lower aromaticity, lower molecular weights, and protein-like signatures. Organic soil horizons had the largest quantities of ESOM, and ESOM decreased with increasing soil depth. ESOM quantities also generally decreased from the upland to the wetland, suggesting that continuous soil saturation leads to a decreased quantity of ESOM. Despite wetland soils having lower ESOM, these horizons are thicker and continuously hydrologically connected to wetland surface

water, leading to wetland soils representing the largest potential source of DOM to the Delmarva Bay wetland system. Knowledge of which soil horizons are most biogeochemically significant for DOM transport in Delmarva and other GIW systems will become increasingly important as climate change is expected to alter the hydrologic connectivity of wetland soils to the surface water-groundwater continuum and as wetlands are more frequently designed for carbon sequestration.

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GENERAL AUDIENCE ABSTRACT

Wetlands store carbon in their plant biomass and soils, which helps to mitigate the effects of climate change by keeping carbon out of the atmosphere. Carbon builds up in wetland soils because the continuously wet conditions slow down the microbial processes that would otherwise break down the organic matter (carbon) and release it to the atmosphere via greenhouse gas emissions. However, the organic matter that accumulates in wetland soils may be released into water, generating dissolved organic matter (DOM). This DOM has the potential to flow out of the wetland, providing a source of energy to aquatic organisms or impacting downstream water quality. Not all wetlands are continuously connected to other water bodies. Geographically Isolated Wetlands (GIW) are wetlands that you could walk all the way around and keep your feet dry. Despite lack of continuous surface water connections, GIWs may still influence downstream water quality via groundwater flow paths or seasonal surface water connections. This variable connectivity makes GIWs a unique setting to study carbon storage and fluxes in wetland soils. The potential for soil-derived DOM generation was studied by extracting organic matter from soils along a wet to dry gradient in Delmarva Bay GIWs. Shallow soils had the largest quantities of extractable soil organic matter (ESOM) and this organic matter is likely sourced from plant inputs to the soil. ESOM from deeper soils was more similar to the microbes that consume and alter the organic matter as it cycles deeper into the soil. Soils located in the wetland basin had less ESOM because continuous saturation depleted the pool of ESOM. Despite lower values of ESOM, wetland soils are

very thick and continuously saturated, making these soils the largest potential contributor of soil-derived DOM to Delmarva Bay GIWs. This work furthers our understanding of how hydrology drives carbon cycling in GIWs and will inform wetland restoration efforts designed to create carbon sinks.

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Contents

List of Figures

List of Tables

List of Abbreviations

DOM Dissolved Organic Matter

EOC Extractable Organic Carbon

ESOM Extractable Soil Organic Matter, also known as Water Soluble Organic Matter (WSOM), Water Extractable Organic Matter (WEOM)

FI Fluorescence Index

GIW Geographically Isolated Wetland

HIX Humification Index

SOM Soil Organic Matter

SSR Spectral Slope Ratio

$SUVA_{254}$ Specific Ultraviolet Absorbance at 254 nm

Chapter 1

Introduction

Wetlands provide critical ecosystem services, such as flood control, nutrient retention, pollutant removal, and carbon storage (Nature Geoscience, 2021). In the face of global climate change and landcover change that results in wetland loss, wetlands are being studied for their ability to act as carbon sinks (Mitsch et al., 2013). Whether wetlands behave as carbon sinks or sources is important in the context of wetland management and restoration efforts designed to sequester carbon. The primary carbon reservoirs in wetlands and their soils include plant biomass, microbial biomass, particulate organic matter (POM), dissolved organic matter (DOM), and gaseous end-products (Kayranli et al., 2010). The balance between biological processes (respiration, methanogenesis), chemical processes (oxidation-reduction), and physical processes (sedimentation, sorption) determines whether wetlands behave as carbon sinks or sources.

The hydrologic regime within wetlands exerts a large influence on the biogeochemical processes that control carbon cycling. For example, wetland water depth drives oxygen availability, soil development, and vegetation characteristics in wetland systems, which in turn impacts carbon inputs. Carbon may be exported from wetlands via greenhouse gas emissions, POM, or DOM. The anaerobic conditions created by saturated conditions slow down decomposition rates, enabling organic matter to build up in wetlands, especially in hydric soils (Catalán et al., 2016, Hoyt et al., 2019, Kayranli et al., 2010, Lacroix et al., 2019). This also makes wetland soils large potential contributors of DOM to downstream

aquatic ecosystems (Creed et al., 2003).

DOM is a complex combination of soluble organic molecules derived from plants and microbes that exists on a composition continuum from fresh to degradation products. DOM is operationally defined as the organic matter in solution that passes through a 0.45 μm filter. DOM is generated autochthonously via primary production in aquatic ecosystems and allochthonously via terrestrial plant and soil organic matter (SOM) inputs (Jones and Stanley, 2016). This results in a continuously evolving mixture comprised of newly generated DOM and the by-products of organic matter degradation. While DOM represents a fraction of total organic matter in ecosystems, DOM is mobile and reactive, making it biogeochemically significant (Jones and Stanley, 2016, Kalbitz et al., 2000). DOM acts as an energy source in aquatic ecosystems and influences microbial nutrient cycling. Additionally, DOM may cause the formation of disinfection by-productions during drinking water treatment, posing a threat to human health. In the context of carbon cycling, if a wetland is exporting large amounts of DOM, the wetland may be a net source of carbon to downstream aquatic ecosystems.

SOM represents a source of DOM to aquatic ecosystems, making it important to understand the reactions and processes that generate soil-derived DOM. Soil-derived DOM movement is influenced by soil moisture, soil mineralogy and texture, redox status, pH, and organic matter quality. Anoxic conditions in saturated soils cause a rise in pH as minerals, like iron and aluminum, are reduced, which favors DOM release (Grybos et al., 2009, Knorr, 2013, Rouwane et al., 2018). Soil DOM concentration decreases and DOM becomes increasingly processed as it moves down the soil profile, typically transitioning from plant-derived molecules to microbial-derived molecules (Kaiser and Kalbitz, 2012, Müller et al., 2009, Roth et al., 2019).

Headwater catchments with wetland land cover have been shown to increase the

amount of DOM exported to river systems (Birkel et al., 2020, Fellman et al., 2009, Hosen et al., 2018, Inamdar et al., 2012). For example, wetland land cover is a predominate explanatory variable in predicting DOM export in Southeast Alaskan watersheds (Fellman et al., 2009). Wetlands may experience continuous or variable hydrologic connectivity, which is defined by the water mediated transport of matter, energy, or organisms within or between elements of the hydrologic cycle (Pringle, 2003). Variation in hydrologic connectivity can create wetting and drying regimes that foster greater diversity in biogeochemical processes (Marton et al., 2015). For example, rewetting of previously dried soil may stimulate decomposition of organic substrates and release of DOM (Borken et al., 1999, Kalbitz et al., 2000, Rouwane et al., 2018). Soils that experience an increased number of fluctuations between saturated and unsaturated conditions (i.e., anaerobic versus aerobic) are hotspots for organic matter decomposition (Capps et al., 2014, Reddy and Patrick, 1975).

Geographically isolated wetlands (GIW) experience variations in hydrologic connectivity, both locally at the aquatic-terrestrial interface and at the catchment scale, because they are completely surrounded by upland and lack a continuous connection to surface water bodies (Tiner, 2003). However, GIWs may be hydrologically connected to other water bodies via groundwater flow paths or periodic surface connections during storm events, allowing for the transport of nutrients and organic matter to downstream water bodies. The terrestrial landscape surrounding GIWs also experiences variable hydrologic connectivity as the groundwater table rises and falls seasonally and as a GIW's spatial extent of inundation expands and contracts throughout the year. Increased hydrologic connectivity leads to lack of processing time for reactions to occur as organic matter is exported from a wetland system, while decreased connectivity leads to increased organic matter processing when organic matter is not able to rapidly move through the wetland system (Covino, 2017). As such, GIWs may have higher DOM concentrations when organic matter accumulates as a result of fewer

flushing events during times of low connectivity but lower DOM concentrations during times of high connectivity where water has less contact time in the soil profile (Jeanneau et al., 2020, Schiff et al., 1998). Soils that experience long durations of inundation see continuous flushing and dilution of DOM. This leads to lower observed DOM concentrations, such as during spring snowmelt (Kalbitz et al., 2000). Whereas, organic matter accumulation and processing occurs during seasonal groundwater table draw down, such as during the summer months, and DOM is then produced when the groundwater table rises again, intersecting previously unsaturated organic soil horizons (Fellman et al., 2008). Despite their variable hydrologic connectivity, GIWs have been shown to influence downstream water quality (Lane et al., 2018, Villa and Bernal, 2018).

Delmarva Bays are a GIW system located on the Delmarva Peninsula in the Mid-Atlantic region of the United States. These GIWs have low-relief topography, soils with low hydraulic conductivity, and a historically forested landscape (Lee et al., 2020). It is estimated that there are 17,000 Delmarva Bays on the Peninsula, however only 29 percent retain natural vegetative cover as the bays have been impacted by agriculture and historical ditching (Fenstermacher et al., 2014). Delmarva Bays alternate between gaining in the spring and losing conditions in the fall (Phillips and Shedlock, 1993), and previous studies have documented the variable surface connectivity of Delmarva Bays to local streams (Epting et al., 2018, Lee et al., 2020, 2019). These surface water-groundwater interactions also influence water chemistry in Delmarva Bays where groundwater collected near the edge of Delmarva Bays shares similar characteristics to the surface water, such as low pH, decreased alkalinity, and high dissolved aluminum concentrations (Phillips and Shedlock, 1993). The concentration and composition of DOM varies between forested, agriculturally impacted, and restored Delmarva Bays, where DOM from forested wetlands is less bioavailable than the protein-like DOM from agricultural and restored wetlands (Hosen et al., 2018).

Extensive work has been done to understand soil carbon storage, methane-cycling microbial communities, greenhouse gas emissions, and seasonal DOM patterns in Delmarva Bays (Fenstermacher, 2012, Hondula et al., 2021, Hosen et al., 2018, Kottkamp, 2019, Maietta et al., 2020). However, there is a knowledge gap related to understanding what DOM sources contribute to resulting surface water DOM dynamics, greenhouse gas emissions, and catchment DOM export. This includes focusing on the hydrologic connectivity of the terrestrial landscape to the aquatic system and studying processes at the soil-water interface to better understand coupled hydrologic and DOM fluxes. Extractable soil organic matter, known as ESOM, quantifies the potential DOM export from soils (Gabor et al., 2015, Jones and Willett, 2006, Rennert et al., 2007). There are no existing studies that have utilized ESOM to measure potential DOM export in wetland soils and to understand how net hydrologic conditions influence ESOM composition. The optical properties of ESOM, assessed through methods such as Excitation-Emission Matrices and PARAFAC modeling, provide broad information about the source, biogeochemical reactivity, and composition of DOM. Pairing ESOM with composition analyses is a useful tool for exploring ecosystem processes that influence the pool of potentially mobile SOM and its fate in downstream waterbodies. To explore soil-derived DOM sources in Delmarva Bay GIWs, the following research questions were explored: (1) What is the quantity and quality of ESOM along an upland to wetland gradient? (2) What is the potential DOM release from Delmarva Bay soils along an upland to wetland gradient? (3) Which soil horizons on the Delmarva Bay landscape are most significant as potential sources of DOM? Wetland soils were hypothesized to have the largest quantities of ESOM and that this ESOM would be more aromatic relative to ESOM from upland soils because saturated wetland conditions slow down decomposition processes, allowing for the accumulation of large organic matter stocks. Wetland soils were anticipated to represent the largest potential source of soil-derived DOM because they are continuously hydrologically connected to Delmarva Bay surface water and groundwater.

Chapter 2

Review of Literature

2.1 Introduction

Wetlands provide critical ecosystem services, such as flood control, nutrient retention, pollutant removal, and carbon storage (Nature Geoscience, 2021). In the face of global climate change, wetlands are being studied for their ability to act as carbon sinks (Mitsch et al., 2013). Whether wetlands behave as carbon sinks or sources is important in the context of wetland restoration efforts designed to sequester carbon. The primary carbon reservoirs in wetlands and their soils include plant biomass, microbial biomass, particulate organic matter, dissolved organic matter, and gaseous end-products (Kayranli et al., 2010). Wetland carbon sequestration is controlled by biological processes (respiration, methanogenesis), chemical processes (oxidation-reduction), and physical processes (sedimentation, sorption). The balance between carbon input and removal mechanisms ultimately leads to increasing carbon stocks.

The hydrologic processes within wetlands are a large control on carbon cycling. For example, wetland water depth drives oxygen availability, soil development, and vegetation characteristics in wetland systems, which in turn impact carbon inputs. The anaerobic conditions created by saturated conditions slows down decomposition rates, enabling organic matter to build up in wetlands, especially in hydric soils (Kayranli et al., 2010). Wetlands may experience continuous or variable hydrologic connectivity, which is defined as the wa-

ter mediated transport of matter, energy, or organisms within or between elements of the hydrologic cycle (Pringle, 2003). Variation in hydrologic connectivity can create wetting and drying regimes that foster greater diversity in biogeochemical processes (Marton et al., 2015).

Dissolved organic matter (DOM) is a wetland carbon reservoir that plays a large role in aquatic carbon cycling. DOM is comprised of soluble organic molecules derived from plants and microbes that exist on a composition gradient from fresh to degradation products. DOM is operationally defined as the organic matter in solution that passes through a 0.45 μm filter. DOM is generated autochthonously via primary production in aquatic ecosystems and allochthonously via terrestrial plant and soil organic matter inputs (Jones and Stanley, 2016). While DOM represents a fraction of total organic matter in ecosystems, DOM is mobile and reactive, making it biogeochemically significant (Jones and Stanley, 2016, Kalbitz et al., 2000). DOM acts as an energy source in aquatic ecosystems and influences microbial nutrient cycling. Additionally, DOM may cause the formation of disinfection by-productions during drinking water treatment, posing a threat to human health. In the context of carbon cycling, if a wetland is exporting large amounts of DOM, the wetland may be a net source of carbon to downstream aquatic ecosystems.

SOM represents a source of DOM to aquatic ecosystems, making it important to understand the reactions and processes that generate soil-derived DOM. Soil-derived DOM movement is influenced by soil moisture, soil texture and structure, landscape setting, and storm events. Movement of DOM through the soil profile allows for microbial processing and physical or chemical removal, resulting in decreased DOM quantity along with changing DOM composition signatures as it cycles downward (Kaiser and Kalbitz, 2012). Saturated wetland soils allow for organic matter to accumulate, making wetland soils large potential contributors of DOM to downstream aquatic ecosystems (Creed et al., 2003, Villa and Bernal,

2018).

Geographically isolated wetlands (GIW) experience variable hydrologic connectivity to downstream water bodies. Varied wetting and drying regimes in GIWs influence resulting soil moisture, redox potential, and microbial processes. These reactions play a role in the mobilization and transformation of the organic matter stored in wetland soils. The objectives of this literature review are to (1) discuss the hydrology of GIWs and previous research in Delmarva Bays, (2) investigate controls on soil-derived DOM mobilization, (3) identify previous studies of DOM export from wetland dominated landscapes, (4) describe methods used to study DOM concentration and composition, and (5) identify knowledge gaps and future areas of study.

2.2 GIWs and their Hydrology

Since hydrologic connectivity influences carbon cycling and biogeochemical processes in wetlands, it is important to consider wetlands that experience variations in hydrologic connectivity, such as geographically isolated wetlands (GIW). GIWs are wetlands completely surrounded by uplands and lack a continuous connection to surface water bodies (Tiner, 2003). However, GIWs may be hydrologically connected to other water bodies via groundwater flow paths or periodic surface connections during storm events, allowing for the transport of nutrients and organic matter to downstream water bodies.

Hydrologic regime is a term used to capture spatial and temporal variations in inundation and soil saturation at wetland sites, including alternating wet and dry regimes, shallow versus deep groundwater flow paths near wetlands, and seasonal water drawdown from evapotranspiration (ET). GIWs may act as temporary storage, sinks, or sources of water and solutes to downgradient water bodies where water fluxes are dependent on frequency,

magnitude, timing, duration, and rate of hydrologic events (Rains et al., 2016). GIWs experience complex groundwater-surface water interactions where GIWs may be gaining during cold seasons when ET is low, losing during warm seasons when ET is high, or flow-through systems relative to the groundwater table. These interactions are dependent on factors such as climate, geology, and GIW basin morphology (Neff et al., 2020). From a landscape perspective, the presence of GIWs creates complex groundwater flow paths (Neff et al., 2020). Surface water-groundwater interactions may control regional hydrology where landscapes an increased number of small wetlands act to buffer surficial aquifer dynamics and baseflow variations (McLaughlin et al., 2014).

2.3 Delmarva Bays and Previous Research

Delmarva Bays are a GIW system located on the Delmarva Peninsula in the Mid-Atlantic region of the United States. These GIWs have low-relief topography, soils with low hydraulic conductivity, and a historically mostly forested landscape (Lee et al., 2020). Precipitation is fairly uniform across wet and dry seasons with an average annual rainfall of 1200 mm (Lee et al., 2020). It is estimated that there are 17,000 Delmarva Bays on the Peninsula; however, only 29 percent retain natural vegetative cover as the bays have been impacted by agriculture and historical ditching (Fenstermacher et al., 2014). Previous research has characterized Delmarva Bay morphology, hydrology, soil organic carbon (SOC) stocks, surface water DOM dynamics, methane emissions, and microbial communities.

Delmarva Bays exhibit fill-spill-merge hydrology, where wetlands will merge during wet seasons and will spill if their storage capacity is reached (Lee et al., 2020). Delmarva Bays alternate between gaining in the spring and losing conditions in the fall (Phillips and Shedlock, 1993). During spring wet seasons, GIW water levels recede at a slower rate than

downstream discharge, indicating high potential surface connectivity (Lee et al., 2020). Seasonal wetland to temporary stream connection duration in the Delmarva Bay system ranges from 64 to 298 cumulative days throughout the year, and peak flows are seen in spring when wetland surface water levels are at their highest elevations (Epting et al., 2018). During fall dry seasons, GIW water levels recede at a faster rate than downstream discharge, showing the large draw on water storage from ET. Following summer rain events, surface water connection durations are short, on the magnitude of hours. More than half of the wetland-stream surface connections turned on and off within a three-week period during the 2014-15 study by Epting et al. (2018) showing the homogeneity of surface connections. This variable surface water connectivity influences seasonal DOM patterns. The concentration and composition of DOM varies between forested, agriculturally impacted, and restored Delmarva Bays (Hosen et al., 2018). DOM concentrations peak during the fall and winter months when Delmarva Bays have surface water connections and DOM from forested wetlands is less bioavailable than the protein-like DOM from agricultural and restored wetlands (Hosen et al., 2018).

Delmarva Bay hydrology is heavily influenced by interactions with groundwater. Soil hydraulic conductivity controls the extent of vertical gradients between Delmarva Bay surface water and groundwater. Wetlands with high conductivity soils act to infiltrate water to the local groundwater system, thus sustaining water holding capacity; whereas, wetlands with low conductivity soils are more likely to fill and spill (Lee et al., 2019). These surface water-groundwater interactions also influence water chemistry in Delmarva Bays. Groundwater collected near the edge of Delmarva Bays shares similar characteristics to the surface water, such as low pH, decreased alkalinity, and high dissolved aluminum concentrations. Shallow groundwater chemistry may impact surfacewater bodies, but as groundwater flow path lengths increase, water chemistry evolves to resemble that of the deeper aquifer (Phillips

and Shedlock, 1993).

Previous studies along upland to wetland transects have explored the influence of hydrology on soil organic carbon stocks, methane emissions, and methane-cycling microbial communities in Delmarva Bays (Hondula et al., 2021, Kottkamp, 2019, Maietta et al., 2020). Delmarva Bay SOC stocks decrease with increasing soil depth and decrease from the wetland to the upland (Fenstermacher, 2012, Kottkamp, 2019). Fenstermacher (2012) found that Delmarva Bays impacted by agricultural activities, such as drainage and land use conversion, had decreased SOC stocks, indicating that restoration efforts could aid in increasing carbon sequestration. SOC macroaggregate stabilization mechanisms were more prevalent moving from the upland to the wetland, while organo-mineral associations where highest in transition and upland soils (Kottkamp, 2019). SOC stocks were significantly related to drying metrics, such as minimum water level and summertime recession rate, as opposed to wetting metrics. Studying microbial communities along wetland transects, Maietta et al. (2020) found that shallow soils (0 – 30 cm) closer to the wetland supported a higher relative abundance of methanogens, while upland soils had a more diverse microbial community. This shift in microbial community diversity is due to long-term hydrological trends. This study posits that diversity of methanogen families present will likely allow methanogenesis to proceed even as composition of the dissolved organic carbon and dissolved inorganic carbon pool varies, meaning microbial activity will not be limited by any potential changes to the type of organic matter available in the soil substrate pool. Hondula et al. (2021) found that inundated soils were the dominate source of methane emissions and dry soils acted as a methane sink, likely as a result of the microbial communities identified by Maietta et al. (2020).

2.4 Biogeochemical Controls on Soil DOM Cycling

The controls on DOM cycling are redox status, pH, mineralogy, organic matter quality, and hydrologic regime. DOM cycling studies are a mix of laboratory experiments and sampling at natural wetland sites. This section will discuss each control separately, but it is important to note that these processes are interrelated and co-influence DOM mobilization in wetland soil environments. Because of this, one control usually cannot be considered without acknowledging the presence of others.

2.4.1 Redox Status

The redox status of wetland soils is generally characterized as reducing when there are inundated conditions creating anaerobic environments and oxidizing when there are dry conditions creating aerobic environments. Fluctuations between reducing and oxidizing conditions create environments favorable for DOM release and mineralization of SOM (D'Amore et al., 2010, Reddy and Patrick, 1975, Rouwane et al., 2018). Reducing conditions, indicated by decreased redox potential, have been shown to decrease annual carbon dioxide efflux in mineral wetland soils (Lacroix et al., 2019). These studies show that reducing conditions allow organic matter to build up in the soil. Whereas, oxidizing conditions lead to the mineralization of organic matter, which in turn increases DOM export should soils become saturated.

2.4.2 pH

The pH of the soil environment plays a large role in organic matter mobilization because pH controls chemical processes such as solubility of ions in solution and protona-

tion/deprotonation of mineral surfaces, which in turn can release or adsorb organic matter. pH values in wetland soils may be controlled by parent material where sandstone provides less of a buffer to pH than limestone (Thompson et al., 2009). Low pH values promote strong DOM adsorption (Kalbitz et al., 2000). However, maximum DOM leaching may occur in a pH range of 4 to 4.5 due to proximity to the dissociation constant of carboxyl functional groups (Evans et al., 2012). pH values below 4 start to constrain the biological activity that helps produce DOM; whereas, once pH increases beyond 4.6, the rate of DOM production became limiting rather than pH dependent solubility (Evans et al., 2012). Evans et al. (2012) suggest that decreased acid deposition could be causing a rise in soil pH, leading to loss in organic horizon carbon. Knorr (2013) showed that acidity can be generated from re-oxidation of reduced species, such as iron (Fe), which may flocculate DOM. Anoxic conditions cause the pH to rise as minerals, like Fe and aluminum (Al), are reduced and release cations, allowing for DOM release (Grybos et al., 2009, Thompson et al., 2009). In the study by Grybos et al. (2009), under oxic conditions where pH was held at higher values, a larger proportion of DOM was released from the SOM pool. These findings suggest that pH is a master variable for organic matter release because it controls reactions on mineral surfaces.

2.4.3 Mineralogy

DOM interacts with mineral surfaces via adsorption, ligand exchange, and surface complexation on clays, Al oxides, and Fe oxides (Kalbitz et al., 2000). Variables such as pH and redox status have significant influences on the behavior of mineral surfaces in wetland soils. For example, in anaerobic or reducing conditions, Fe and Manganese (Mn) are soluble. When Fe(III) is reduced to Fe(II) in anoxic conditions, DOM is released (Chen et al., 2020, Knorr, 2013, Zhao et al., 2020). In transitions from oxic to moderately reducing conditions, Mn and Fe become reduced, leading to a pH increase as adsorbing surfaces expe-

rience dissolution or deprotonation of hydroxyl groups. These mineral surface reactions lead to electrostatic repulsion of DOM molecules and increased DOM release (Rouwane et al., 2018). However, DOM processing by Fe(III) reducing microbes may increase at higher temperatures leading to decreased DOM export from shallow-organic rich alpine wetland soils (Pallud et al., 2020). Because Fe is soluble in reducing conditions, Al may have a greater influence on carbon content in wetland soils (Lacroix et al., 2019). It is suggested that clay minerals only overtake Fe and Al oxides on adsorbing DOM if Fe oxides disappear (Fiedler and Kalbitz, 2003).

When there is a transition from anoxic to oxic conditions, Fe(II) is oxidized, the pH decreases, and, in turn, DOM complexes with the Fe, reducing its export (Knorr, 2013). DOM is retained in aerobic soils when there are lots of Fe oxides (Schiff et al., 1998). Duan et al. (2020) found that Fe-organic matter complexes where higher in the rhizosphere of wetland soils because of increased oxygen availability. It is evident that mineral surfaces play a large role in the adsorption and desorption of organic matter dependent on pH and redox status.

2.4.4 Organic Matter Quality

The quality, or composition, of organic matter refers to the source and chemical structure of the organic matter and how extensively it has been altered through biotic and abiotic processes. Organic compounds decay along a continuum where breakdown of larger, energy rich molecules will lead to decreases in size, potential increases in solubility, and increased opportunity for mineral protection against further decomposition (Lehmann and Kleber, 2015). Many of the aforementioned studies discuss DOM composition, in addition to concentration, as a result of the biogeochemical processes that occur in wetland soils.

Studies agree that DOM concentration decreases and DOM becomes increasingly microbially altered as it moves down the soil profile, typically transitioning from plant-derived molecules to microbial-derived molecules (Kaiser and Kalbitz, 2012, Müller et al., 2009, Roth et al., 2019). Through adsorption processes, there is preferential removal of hydrophobic and high molecular weight fractions of DOM (Kalbitz et al., 2000). Rouwane et al. (2018) showed that changes in redox status involved the release of aromatic substances; whereas, there was low release of protein-like substances because these are preferentially used by microbes. Biomass inputs influence resulting DOM properties along the soil profile. For example, (Lacroix et al., 2019) demonstrated that root biomass increases as you move from wetland into upland areas, altering the type of inputs to the carbon pool. They also showed that wetland surface soils had a greater abundance of high molecular weight and aromatic carbon. The quality of DOM mobilized from SOM will therefore reflect the environmental factors, such as microbial degradation or mineral protection, that influenced the preservation of the SOM.

2.4.5 Hydrologic Connectivity

Landscapes with wetland land cover have been shown to increase DOM exported to river systems (Birkel et al., 2020, Fellman et al., 2009, Hosen et al., 2018). Hydrologic connectivity and residence time regulate water quality. Increased connectivity leads to lack of processing time for reactions to occur as organic matter is exported through a wetland system, while decreased connectivity leads to increased organic matter processing when organic matter is not able to rapidly move through the wetland system (Covino, 2017). As such, GIWs may have higher DOM concentrations due to organic matter accumulation as a result of fewer flushing events during times of low connectivity but lower DOM concentrations during times of high connectivity where water has less contact time in the soil profile (Jeanneau

et al., 2020, Schiff et al., 1998). Many studies show that organic matter decomposition is correlated to water table depth, where a higher water table results in slower decomposition (Catalán et al., 2016, Hoyt et al., 2019, Lacroix et al., 2019). This slower decomposition is often less efficient since it likely occurs during anaerobic conditions, meaning there are higher amounts of water-soluble DOM intermediates being released (Kalbitz et al., 2000).

Studies have found that rewetting of previously dried soil may stimulate decomposition of organic substrates and release of DOM (Borken et al., 1999, Kalbitz et al., 2000, Rouwane et al., 2018). Soils that experience an increased number of fluctuations between saturated and unsaturated conditions (i.e., anaerobic versus aerobic) are hotspots for organic matter decomposition (Capps et al., 2014, Reddy and Patrick, 1975). Soils that experience long durations of inundation see continuous flushing and dilution of DOM, leading to lower observed DOM concentrations, such as during spring snowmelt (Kalbitz et al., 2000). Whereas, organic matter accumulation and processing occurs during seasonal groundwater table draw down, such as during the summer months, and DOM is then produced when the groundwater table rises again, intersecting organic soil horizons (Fellman et al., 2008). These studies demonstrate how hydrologic regime plays a large role in driving oxygen status and resulting DOM transformation and transport in wetland soils.

DOM export processes are similar in other landscape settings where seasonal stream DOM concentration and composition vary due to different flow path depths during storm events and baseflow conditions. Snowmelt and storm events tend to mobilize DOM from shallow soils as the groundwater table rises. This results in higher DOM concentrations and DOM with increased aromaticity and terrestrial signatures (Barnes et al., 2018, Singh et al., 2014). DOM concentrations have been found to increase with the rising limb of the hydrograph or peak after the hydrograph peaks (Boyer et al., 1995, Saraceno et al., 2009, Werner et al., 2019). During baseflow conditions, DOM reflects deeper flow paths with lower

DOM concentration but increased microbial-derived, protein-like fluorescence (Barnes et al., 2018, Shen et al., 2015, Singh et al., 2014).

2.5 Methods in DOM Analysis

2.5.1 DOM Optical Properties, Fluorescence Metrics, and PARAFAC Modeling

DOM is a complex combination of organic compounds that reflect multiple organic matter sources and a serious of alteration or degradation processes. This complex chemical nature makes it difficult to rapidly assess DOM composition. However, the ability to absorb and reflect light provides insights to the composition characteristics of DOM. Both absorbance and fluorescence spectroscopy are techniques used to determine the optical properties of DOM. Absorbance spectroscopy measures the fraction of DOM that absorbs light or is chromophoric, known as CDOM. Fluorescence spectroscopy analyzes the fraction of DOM that both absorbs and re-omits light, known as fluorophores or FDOM. CDOM and FDOM provide broad information about the source, biogeochemical reactivity, and composition of DOM.

Previous studies have developed metrics that relate absorbance and fluorescence data to organic matter characteristics. Specific Ultraviolet Absorbance (SUVA) is the absorbance of samples at a specific wavelength, most commonly 254 nm, normalized to the DOM concentration. $SUVA_{254}$ is correlated to percent aromaticity of DOM, where higher values of $SUVA_{254}$ indicated there are aromatic molecules present (Weishaar et al., 2003). Using whole water samples from a wide range of riverine environments, Weishaar et al. (2003) found that samples with $SUVA_{254}$ values of 5-6 L·mg C^{-1}·m^{-1} were correlated to approximately 35-40%

aromaticity, whereas $SUVA_{254}$ values of 1-2 L·mg C^{-1}·m^{-1} were correlated to approximately 5-15% aromaticity. Notable for a wetland soil focus, this study found that pH levels below 4 and increased ferric iron concentrations influenced the absorbance of samples, where low pH decreased absorbance and higher ferric iron levels increased absorbance.

Similar to $SUVA_{254}$, the Humification Index (HIX) measures the complexity and aromatic nature of DOM (Gabor et al., 2015). HIX is the ratio of emission (435 nm – 480 nm) / (300 – 345 nm) measured at 254 nm excitation (Ohno, 2002, Zsolnay et al., 1999). Higher values of HIX indicate more ring structure and larger molecules, while lower values indicate an increased degree of processing and decreased Hydrogen/Carbon (H/C) ratios. Using soil DOM extracts, Zsolnay et al. (1999) observed HIX values ranging from 2 – 16, where lower values were associated with muck soils that had emissions spectra shifted to lower wavelengths as the result of lower H/C ratios. Spectral Slope Ratio (SSR) is the ratio of (275 nm – 295 nm) to (350 nm – 400 nm) absorbance values and is inversely related to molecular weight of CDOM in samples (Helms et al., 2009). Evaluating SSR along a swamp to coastal gradient, Helms et al. (2009) observed SSR values ranging 0.5 to 2.5, where low SSR values were correlated with high molecular weight DOM. The Fluorescence Index (FI) is the ratio of emission wavelengths at 470 nm and 520 nm at an excitation wavelength of 370 nm (McKnight et al., 2001). The FI identifies the relative contribution of plant and microbial sources in DOM samples. Higher FI values ($\approx$ 1.8) indicate microbial or autochthonous DOM, while lower values ($\approx$ 1.2) indicate plant or allochthonous derived DOM. Collectively, these metrics have been used to classify organic matter composition based on optical properties.

An additional aspect of fluorescence spectroscopy is the ability to generate Excitation-Emission Matrices (EEMs). EEMs allows for the identification of organic compounds with similar composition through the evaluation of peaks in fluorescence values that occur within

a certain range of excitation and emission wavelengths. For example, Coble (1996) identified five primary peaks: A, C, M, B, and T. Peaks A and C are derived from plant sources, have high molecular weight, and are aromatic (Fellman et al., 2010). Peak M is less aromatic and lower molecular weight than A and C. Peaks B and T are associated with tyrosine and tryptophan, respectively (Fellman et al., 2010). These fluorophore peaks are associated with protein-like sources typically derived from microbial sources. EEMs show the fluorescence signature of DOM and can be broken down into the components that make up the fluorescence signature using Parallel Factor Analysis (PARAFAC). Cory and McKnight (2005) generated a 13 component PARAFAC model using a wide range of DOM sources to identify the presence of oxidized quinones, reduced quinones, and amino acid-like signatures in DOM. Researchers can apply this developed PARAFC model to new DOM data sets to determine which previously identified components are prevalent in their samples. Alternatively, researchers may develop their own PARAFAC model to identify unique components in their samples (Murphy et al., 2013b).

2.5.2 Extractable Soil Organic Matter

The potential for DOM generation in soil horizons may be evaluated through soil organic matter extractions, which is advantageous in situations where it is difficult to measure soil DOM in-situ. Known by a number of names such as Water Soluble Organic Matter (WSOM), Water Extractable Organic Matter (WEOM), or Extractable Soil Organic Matter (ESOM), there have been multiple studies performed characterizing ESOM (Table 2.1) from a variety of ecosystems. ESOM can be analyzed for both DOM concentration and composition, providing insights to the quantity and sources of organic matter that have the potential to be mobilized from soil horizons. However, there is no universally accepted method for performing ESOM extraction experiments. The various experimental procedures generally

include the basic steps of sampling soils by horizon, mixing a subsample of soil with an extractant solution (often demineralized water (DIW), $CaCl_2$, K_2SO_4 or KCl) at a fixed ratio, shaking the mixture on a shaker table, centrifuging the well-mixed solution, and filtering the centrifuged solution.

It's important to note that ESOM provides a measurement of the pool of potentially mobile DOM. Because ESOM extraction is a laboratory procedure that destroys intact soil structure, ESOM may contain organic matter that would not naturally enter the dissolved phase because of its location within the soil matrix, and the extraction may cause lysis of living cells. Field moist soil is preferred over air dried or re-wetted air dried soil because drying tends to cause increased ESOM concentration and increased microbial signatures in ESOM (Jones and Willett, 2006, Kaiser et al., 2015). Of the various extractant solutions, K_2SO_4 extracts the most organic matter while $CaCl_2$ extracts the least (Gabor et al., 2015, Rennert et al., 2007). $CaCl_2$ provides a more conservative ESOM estimate because Ca^{2+} ions may cause organic matter to precipitate or adsorb to metal oxides (Cincotta et al., 2019, Rennert et al., 2007). $CaCl_2$ may be a more desirable extractant solution because other solutions increase protein-like signatures in ESOM fluorescence data (Gabor et al., 2015).

Many published ESOM studies have focused on methodology, comparing extraction solutions, ideal temperatures, and soil moisture levels. However, while exploring methods, these studies have been carried out in a variety of landscape settings, such as forested, agricultural, and grassland soils, providing a unique opportunity to synthesize the use of the ESOM procedure across a broad range of soil ecosystems. ESOM has been assessed in the context of lateral soil horizon development (Duston, 2020), biodegradation of terrestrial DOM sources (McDowell et al., 2006), and anticipating the influence soil-derived DOM has on downstream water chemistry (Gabor et al., 2015, Ma et al., 2021). Similar to direct soil DOM measurements, ESOM concentration decreases with increasing soil depth, and ESOM

Table 2.1: Examples of ESOM extraction procedures from previous studies.

Study	Study System	Maximum depth sampled	Extractant Solution(s)	Ratio of Extractant Solution to Soil	Shaking speed & duration	Centrifuge speed & duration
Corvasce et al., (2006)	Forested catchment, Italy	200 cm	0.01 M CaCl2	2:1	Stirred for 10 min	4,000 rpm for 10 min
Duston, (2020)	Hubbard Brook Experimental Forest, New Hampshire, USA	70 cm	0.01 M CaCl2	5:1	Wrist action shaker for 1 hr	2,000 g for 15 minutes
Gabor et al., (2015)	Forest soils of headwater catchment in Colorado, USA.	5 cm	1. Nano pure H_2O 2. 0.5 M K_2SO_4 3. 0.01 M $CaCl_2$ 4. 2 M KCl	5:1	200 rpm for 1 hr	15 min (no speed listed)
Jones and Willett, (2005)	Grassland and woodland soils, United Kingdom	20 cm	1. DIW 2. 2 M KCl	10:1	200 rpm for 15 min	8,000 g for 10 min
Ma et al., (2020)	Forested catchment, China	100 cm	DIW	5:1	6.99 g for 1 hr	694 g for 15 min
McDowell et al., (2006)	Forest and agricultural soils in various locations	10 cm	0.01 M CaCl2	2:1	10 min (no speed listed)	No centrifuge used
Rennert et al., (2007)	Forest soils, Germany	100 cm	1. DIW 2. 0.01 M $CaCl_2$ 3. 0.5 M K_2SO_4	5:1	1 hr (no speed listed)	No details listed

composition is dependent on landscape location (Cincotta et al., 2019, Corvasce et al., 2006, Gabor et al., 2015, Ma et al., 2021). It is common for these studies to assess ESOM composition using absorbance and fluorescence metrics such as $SUVA_{254}$, FI, and HIX, and to decompose the fluorescence signature of ESOM using PARAFAC modeling. Hillslope soils typically have higher FI values indicative of microbial processes that influence the organic matter pool as compared to riparian zone soils where lower FI values reflect an organic matter pool influenced by plant inputs (Cincotta et al., 2019, Gabor et al., 2015). ESOM composition typically transitions from plant-like to microbial-like (seen by increasing FI values), from higher aromaticity to lower aromaticity (indicated by decreasing $SUVA_{254}$ and decreasing HIX) with depth in the soil profile (Corvasce et al., 2006, Duston, 2020, Ma et al., 2021). With the recent soil science paradigm shift in describing "humic substances", caution is recommended when applying previously denoted aquatic organic matter descriptors, such as "humic-like", to ESOM analyses, as these descriptors don't meaningfully represent the abiotic and biotic processes influencing organic matter quality (Lehmann and Kleber, 2015). Whether ESOM becomes mobilized and transported to downstream water bodies depends

on the site's hydrologic conditions and the biogeochemical and physical processes that may promote organic matter retention in the soil profile. Pairing ESOM with composition analyses is a useful tool for exploring ecosystem processes that influence the pool of potentially mobile SOM and its fate in downstream waterbodies.

2.6 Conclusions and Future Work

Through this literature review, there were certain areas of study that elicit further research. There are many studies looking at the seasonality of DOM fluxes in headwater catchments and stream but fewer that look at wetland dominated landscapes. Previous work in the Delmarva Bay system has explored the seasonal patterns in DOM concentration and composition in surface waters, but less is known about the sources contributing DOM to the wetland surface water. Hosen et al. (2018) suggested that more information is needed related to how subsurface hydrologic connections influence DOM dynamics. Additionally, studies of the SOM stocks along upland to wetland transects in Delmarva Bays have determined stabilization mechanisms for soils experiencing variable hydrologic conditions (Kottkamp, 2019). Therefore, it would be beneficial to understand what conditions promote DOM mobilization from SOM stocks and how mobilized DOM may contribute to resulting surface water DOM dynamics and greenhouse gas emissions. This includes studying processes at transition zones and the soil-water interface to better understand coupled hydrologic and DOM fluxes. There have been few studies that utilize ESOM extractions to study potential DOM export in wetland soils and to understand how net hydrologic conditions influence ESOM composition. A standard method for ESOM extractions has yet to be determined. Applying EEMS and PARAFAC modeling to ESOM is a relatively new application of these tools since they were originally developed for aquatic ecosystem samples. Additionally, in-

situ studies would provide better insight to the processes occurring as compared to laboratory studies, which were the bulk of studies in this review, because laboratory studies remove the inherent variability and characteristics of the natural soil environment.

This literature review discussed the major controls on DOM cycling in wetland soils. DOM export is controlled both biotically and abiotically. Each of the studies demonstrated that redox status, pH, hydrologic regime, mineral interactions, and organic matter quality are all significantly interrelated. Shallow subsurface soils are hotspots for DOM mobilization. Soils that experience aerobic and oxidizing conditions will mineralize organic matter, which can then be exported once saturation occurs. Soils that experience an inundated hydrologic regime are likely to be anaerobic and have reducing conditions. This leads to the solubilization of metal oxides and a rise in pH, which increases the potential release mechanisms for DOM. Tying all these controls together shows multiple pathways for DOM mobilization in wetland soils.

The biogeochemical processes discussed here are imperative to understanding the impact GIWs can have on water quality, aquatic metabolism, and ecosystem services. GIWs have been at the forefront of recent debates about which water bodies should be designated for federal protection as Waters of the United States by the Clean Water Act. In order to protect these wetland ecosystems, GIWs must exert a significant nexus of influence on the landscape (Cohen et al., 2016). Additionally, as the climate changes, it will be more important to understand the controls on carbon export from wetland systems as increased warming leads to more precipitation in winter months, higher ET in the summer, and increased draining, all of which could increase carbon mobilization and mineralization. Exploring DOM mobilization and transformation from Delmarva Bay wetland soils has the potential to further demonstrate the significant nexus of influence that GIW systems exert on the landscape.

Chapter 3

Methods

3.1 Study Site

The Delmarva Bay GIWs used in this study are located on the Delmarva Peninsula in the Mid-Atlantic region of the United States (Figure 3.1). The Delmarva Peninsula has a humid, temperate climate with a 30-year normal annual mean precipitation (1980-2010) of 1106 mm and precipitation that is evenly distributed throughout the year (PRISM Climate Group, 2004). The 30-year normal annual mean temperature (1980-2010) is 13.4 °C (PRISM Climate Group, 2004). Four Delmarva Bay wetlands with similar soils, vegetation, and topography were selected for this study. These sites represent the typical Delmarva Bay morphology characterized by an ellipsoidal shape with sandy upland rims (McDonough et al., 2015). The study sites are located within the Choptank River watershed on forested property managed by The Nature Conservancy. The dominant soil map unit is Hammonton-Fallsington-Corsica complex with a parent material of loamy fluviomarine sediments (Soil Survey Staff, 2017).

3.2 Soil Sampling

At each wetland site, an upland to wetland transect of approximately 25 - 45 m in length from wetland center had previously been established by Kottkamp (2019). Along these

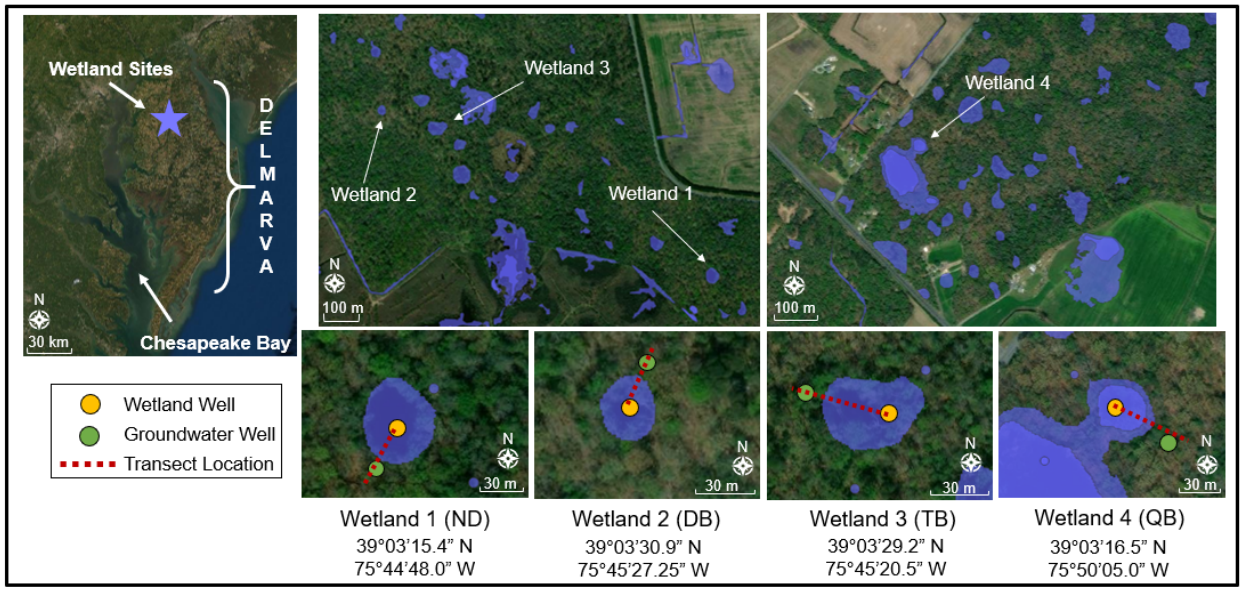

Figure 3.1: Location of study sites on the Delmarva Peninsula.

transects, four equally spaced sampling points (Upland, Transition, Edge, and Wetland) were selected to capture the full range of hydrologic conditions along the aquatic-terrestrial interface (Figure 3.2). For example, the Wetland point was located in the basin area that experiences nearly continuous inundation whereas, the Upland point was located where soils are rarely saturated in the upper 50 cm of the soil profile. Soils were sampled once in the spring and once in the autumn of 2020. At the Upland, Transition, and Edge points, a small soil pit was dug (approximately 30 x 30 cm and 50 cm deep) in order to classify and measure the depth of each horizon. Standing water at certain Wetland and Edge locations prevented the digging of a soil pit. At each transect point, a one-inch diameter push probe was used to collect soil samples to an approximate depth of 40 cm. Each sample typically captured the O (organic), A (topsoil), and portions of the B (subsoil) within the 40 cm sample length. However, the standing water at Wetland and Edge locations prevented samples from reaching as deep as Transition and Upland samples. After confirming that no compaction occurred, soil horizons were separated and bagged by horizon. Leaf litter samples were

collected at each of the four sites during the spring sampling campaign, which occurred prior to the growing season. During both soil sampling events, wetland surface water and upland groundwater samples at each site were filtered into pre-combusted 40 mL amber vials using an acid washed plastic syringe and pre-combusted 0.7 μm Whatman GF/F filters. Leaf litter, surface water, and groundwater samples were collected in addition to soil samples for the purpose of comparing DOM end-members along the Delmarva Bay aquatic-terrestrial continuum. All samples were stored on ice and then in a refrigerator at 4 °C until laboratory analysis was performed.

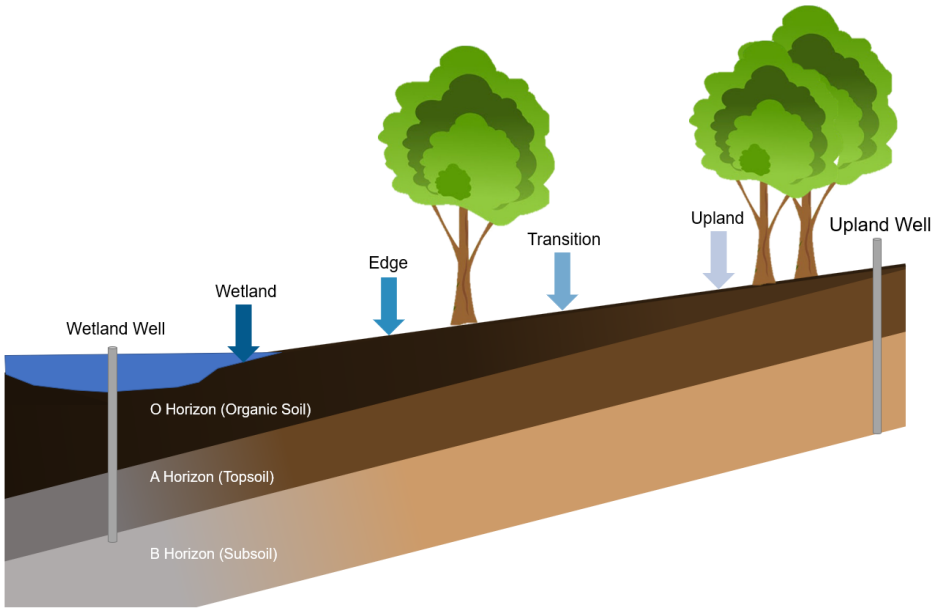

Figure 3.2: Conceptual transect design with four points (Wetland, Edge, Transition, and Upland) in-between the wetland center and upland groundwater monitoring wells. Three soil horizons (O, A, and B) are present along the transect.

3.3 Water Level Monitoring

Continuous water level data, averaged to a daily time-step, was collected over the 2020 water year (October 1, 2019 to September 30, 2020) using HOBO pressure transducers

in previously established wetland and upland monitoring wells. To determine if the 2020 water year was representative of typical water year conditions, long-term discharge data from the Choptank River (USGS 01491000) was utilized to calculate the annual mean daily discharge from 2010-2020. Due to the relatively small spatial scale and low-relief landscape setting of Delmarva Bays, water table elevation along the transects was estimated assuming a linear groundwater table in-between the two monitoring wells. Water level data were used to calculate minimum, maximum, median, and mean water level relative to the ground surface at each transect point. Additionally, the water level data were used to calculate duration of saturation and number of saturation events for each soil horizon at each transect point. Because Wetland and Edge samples only captured a few centimeters of the B horizon, a bottom horizon elevation of -0.5 m was assumed to ensure saturation duration and number of saturation events could be calculated in these horizons. Duration of saturation was determined by summing the number of days the water elevation was above the bottom elevation of each soil horizon. Number of saturation events was calculated by summing the number of times the water elevation moved from below to above the bottom elevation of each soil horizon. A low number of saturation events reflects locations that are rarely inundated, such as the Transition and Upland, meaning the water table does not frequently enter the soil horizons sampled. A low number of saturation events can also reflect more permanent inundation, such as the Wetland or Edge transect points, where standing water above the ground surface means soils are consistently saturated.

3.4 Extraction Procedure

While there is no universally accepted soil organic matter extraction procedure, methodology for this experiment was developed utilizing methods from Chantigny (2003), Duston

(2020), Gabor et al. (2015), Jones and Willett (2006), McDowell et al. (2006), and Rennert et al. (2007). During the date of extraction, field moist soil samples were removed from the refrigerator and sorted to remove any large rocks, roots, and debris. 30 g of field moist soil was mixed with 150 mL of 0.01 M Calcium chloride in a flask. Calcium chloride was selected as the extractant solution because it provides a conservative estimate of the SOM that could enter the dissolved phase (Gabor et al., 2015, Rennert et al., 2007). The flasks were then placed on a shaker table for one hour at a speed of 200 rpm at room temperature (25 °C). An additional 15 g of soil was measured out and placed in an oven at 110 °C for 24 hours to determine moisture content of each soil horizon. Immediately following shaking, the well-mixed soil-extract solution was divided into two 50 mL Falcon centrifuge tubes and centrifuged at 4,000 rpm for 10 minutes. Centrifuged solution was then filtered into two pre-combusted 40 mL amber vials using an acid washed plastic syringe and pre-combusted 0.7 μm Whatman GF/F filter. The vials were stored in a refrigerator at 4 °C until further analysis. Soil texture was determined using the hydrometer method (Bouyoucos, 1936, Day, 1950, 1953). Spring leaf litter samples from each of the four sites were also extracted using this procedure.

3.5 Instrumental Analyses and PARAFAC Modeling

Filtered extracted soil solution, extracted leaf litter solution, surface water, and groundwater samples were analyzed for Non-Purgeable Organic Carbon and Total Dissolved Nitrogen on a Shimadzu TOC-Vcph Carbon Analyzer with TNM-1 Total Nitrogen Unit. Nitrate concentrations were measured on a Dionex ICS-3000 Ion Chromatography System. Samples were analyzed for absorbance on a Shimadzu UV Spectrophotometer immediately followed by fluorescence analysis on a Horiba FluoroMax-4 Spectrofluorometer. The spec-

trophotometer collected absorbance data from 190 to 850 nm in 1 nm increments and samples were blank corrected during post-processing. To reduce inner-filter effects during fluorescence analysis, samples with absorbance values greater than 0.2 cm^{-1} at 240 nm were diluted so that they fell within 0.02 – 0.2 cm^{-1}. Excitation-Emission Matrices (EEMs) were collected with an excitation wavelength range of 240-450 nm in 5 nm increments and emission wavelength range of 300-600 nm in 2 nm increments. During post-processing, EEMs were corrected for inner-filter effects, Raman normalized, and blank corrected. Fluorescence metrics such as Specific Ultraviolet Absorbance ($SVUA_{254}$), Fluorescence Index (FI), Humification Index (HIX), and Spectral Slope Ratio (SSR) were calculated after post-processing of absorbance and fluorescence data in order to classify ESOM characteristics. $SUVA_{254}$ normalizes absorbance at 254 nm to ESOM concentration where higher values indicate increasing aromaticity of organic matter (Weishaar et al., 2003). FI is the ratio of emission at wavelengths 470 nm and 520 nm at an excitation wavelength of 370 nm and identifies the relative contribution of plant and microbial sources in DOM samples (McKnight et al., 2001). Higher FI values ($\approx$ 1.8) indicate microbial or autochthonous DOM, while lower values ($\approx$ 1.2) indicate plant or allochthonous derived DOM. HIX is the ratio of emission (435 nm – 480 nm) / (300 – 345 nm) measured at 254 nm excitation and measures the complexity and aromatic nature of DOM (Gabor et al., 2015, Ohno, 2002, Zsolnay et al., 1999). Higher values of HIX indicate more ring structure and larger molecules, while lower values indicate an increased degree of processing and decreased Hydrogen/Carbon ratios. Spectral Slope Ratio (SSR) is the ratio of (275 nm – 295 nm) to (350 nm – 400 nm) absorbance values and is inversely related to molecular weight of DOM in samples (Helms et al., 2009).

Parallel factor analysis (PARAFAC) decomposes the fluorescence signature of EEMs to identify the underlying organic matter signals (Murphy et al., 2013b). Using MATLAB ver. 2019b, the Cory and McKnight PARAFAC model was employed to assess ESOM sam-

ple loadings on the previously identified thirteen components (Cory and McKnight, 2005). ESOM sample loadings were also analyzed on a four component PARAFAC model developed using surface water and groundwater samples from approximately twenty Delmarva GIWs and two end-member rivers collected bi-monthly during the 2020 water year. This PARAFAC model was created using the drEEM toolbox (Murphy et al., 2013a,b). The four components were uploaded to the OpenFluor database to elucidate potential organic matter sources from other PARAFAC models that have produced similar fluorescence signatures (Murphy et al., 2014).

3.6 Estimating Potential DOM Release

ESOM quantities at the time of sampling provide an estimate of the potential DOM that may be exported if soils were to become saturated. Whether or not DOM is actually mobilized is a function of saturation event characteristics such as duration of saturation or number of saturation events. Saturation events influence redox status and mineral solubility and therefore the rate of DOM release. These saturation event characteristics vary depending on location along the transect, where upland soils are rarely saturated while wetland soils are nearly constantly saturated. Previous DOM release studies have observed first order decay trends where DOM release starts high when soils are first saturated, decreases over time, and ultimately levels out (Chow et al., 2006, Christ and David, 1996). To estimate and compare the DOM that would be released from Upland O horizons and Wetland O horizons, a first order decay function was applied to ESOM results:

$$C = C_0 * e^{-kt} \tag{3.1}$$

where C is the mean O horizon ESOM quantity averaged across all four sites at time of spring sampling (EOC, g EOC/m^2), C_0 is the theoretical initial O horizon ESOM quantity (EOC, g EOC/m^2) available for release prior to any saturation, k is a decay constant (1/day) estimated from previous DOM release studies (Chow et al., 2006, Christ and David, 1996), and t is the mean duration of saturation (day) from the start of the 2020 water year to the date of spring soil sampling across all four sites.

Upland and Wetland O horizons were the only horizons chosen for this analysis because they represent the two hydrologic extremes on the landscape, where Wetland O horizons were continuously saturated and Upland O horizons were continuously dry during the period of analysis. Mean spring ESOM quantities were reported per square meter by multiplying ESOM results by mean horizon thickness and mean horizon bulk density. Bulk density values were assigned to horizons using soil bulk density data reported along Delmarva Bay transects by Kottkamp (2019). The ESOM quantity at the time of sampling was used as C, allowing C_0 to be back calculated. In cases where there were zero days of saturation, such as the Upland O horizon, $C = C_0$. A decay constant of 0.01^{-d} was assumed by comparing parameters from nonlinear regression (nls) models fitted in R using data from DOM release studies on agricultural peat soils (Chow et al., 2006) and forested soils (Christ and David, 1996). Duration of saturation from the start of the 2020 water year (October 1, 2019) to the date of spring soil sampling (March 10, 2020) was calculated by summing the number of days the water elevation was above the bottom elevation of the Upland and Wetland O horizons. This period of saturation was selected because the 2020 water year started out dry and then continuously wetted up through the spring. This allowed the saturation duration calculation to capture one continuous saturation event. This decay model assumes no additional accumulation or mineralization of organic matter.

3.7 Statistical Analyses

All statistical analyses were performed in R ver. 4.1.0 (R Core Team, 2021). Paired t-tests were used to verify that ESOM results and PARAFAC sample loadings were consistent between the spring and autumn sampling campaigns. Analysis of Variance (ANOVA) paired with Tukey Honest Significance Difference (HSD) tests were used to determine if minimum, mean, median, and maximum water level and ground elevation are significantly different between transect points during the 2020 water year. ANOVA and Tukey HSD tests were also performed to evaluate differences in ESOM concentration and composition based on (1) soil horizon and (2) transect position for each sampling campaign. The normality of ANOVA model residuals was evaluated using the Shapiro-Wilks test ($\alpha = 0.05$) and equal variance was evaluated using the Bartlett, Levene, or Brown-Forsythe tests ($\alpha = 0.05$). If residual normality assumptions were not met, a non-parametric Kruskal-Wallis test was performed in place of an ANOVA. Analysis of Covariance (ANCOVA) was used to determine if there was a significant interaction between both soil horizon and transect location. K-means cluster analysis was performed to determine if ESOM results would be divided into groups based on soil horizon. Cluster analysis was performed using the cluster and factoextra packages in R (Kassambara and Mundt, 2020, Maechler et al., 2021). The average silhouette method was used to determine the optimal number of clusters.

Chapter 4

Results

4.1 Soil Classification

Along the transects, mean O horizon thickness increased from 7.8 cm in the Upland to 18 cm in the Wetland (Figure 4.1). O horizons from all transect points had significant organic matter debris and roots, preventing texture analysis from being performed. However, organic O horizons at the Wetland and Edge points visually appeared to be sapric, while Transition and Upland O horizons appeared to be fibric. Regardless of transect point, A and B horizon soil textures were loamy sand or sandy loam. In Wetland and Edge points where the B horizon was captured during sampling, B horizon soils displayed depleted matrix colors, indicative of hydric soils (NRCS, 2006). At Wetlands 3 and 4, the B horizons at the Transition point had darker matrix colors compared to Wetlands 1 and 2 (Appendix Figures A.1, A.2 and A.3).

4.2 Wetland Hydrology

The Choptank River mean daily discharge for the 2020 water year was above the 2010-2020 overall median daily flow (Figure 4.2). Therefore, the 2020 water year was considered a wet year for Delmarva Bay site hydrologic conditions. The 2020 water year started dry, with water levels falling near or below -0.5 m across all four transect points at all four wetland

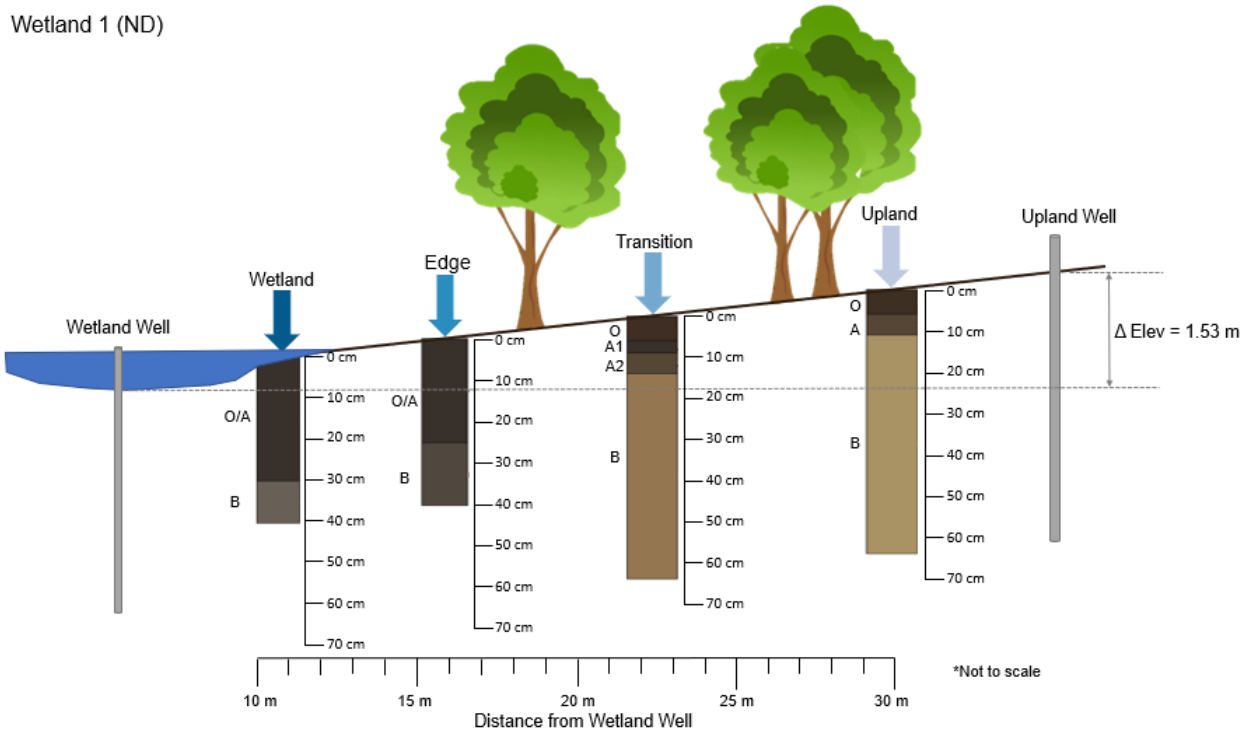

Figure 4.1: Soil horizon depths (y-axis) and distance from wetland center along the transect (x-axis) at Wetland 1.

sites (Appendix Figure A.8). Water levels then rose during the late winter and remained elevated throughout the spring months. Water levels then experienced large fluctuations during summer storm events but remained elevated through the end of the 2020 water year.

Considering the average hydrologic conditions across all four sites, the water table at the Wetland, Edge, and Transition points fluctuated in and out of the soil sampling zone while the Upland point remained unsaturated throughout the entire 2020 water year (Figure 4.3 Panel A). The Wetland point was the only transect spot that had a mean water elevation above the ground surface (Figure 4.3 Panel B, Table 4.1). The mean water level and mean number of days saturated at or above the upper 0.5 m of the soil profile increased moving from the Upland to the Wetland (Figure 4.3 Panel B and C, Table 4.1). The Wetland and Edge points were consistently wet while the Upland was consistently dry, leading to few

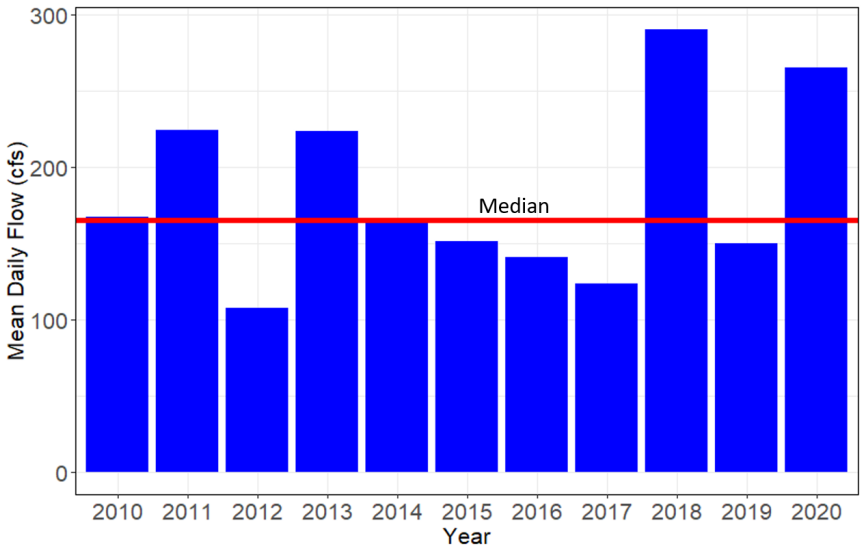

Figure 4.2: Mean daily flow (cfs) on the Choptank River from 2010-2020.

alternations between saturated and unsaturated conditions in the upper 0.5 m of the soil profile in these positions, whereas the Transition point at each of the sites experienced a mean of 6.5 ± 2 saturation events (Figure 4.3 Panel D, Table 4.1).

The O and A horizons in the Wetland and Edge points across all four sites were inundated over 200 days while the Transition and Upland horizons were never (O horizons) or rarely (A horizons) saturated. O horizons had a higher mean number of saturation events in the Wetland (1.5 ± 1.0) and Edge points (2.5 ± 1.2), while the Transition and Upland O horizons each had zero events. B horizons at the Transition location had the largest mean number of saturation events (6.5 ± 1.3) (Table 4.2, Appendix Figure A.4).

4.3 Extractable Soil Organic Matter

Soil sampling occurred when water levels were elevated in the spring due to low evapotranspiration demands and again in autumn when water levels were high due to summer storm events. Paired t-tests demonstrated that ESOM quantity and quality results were

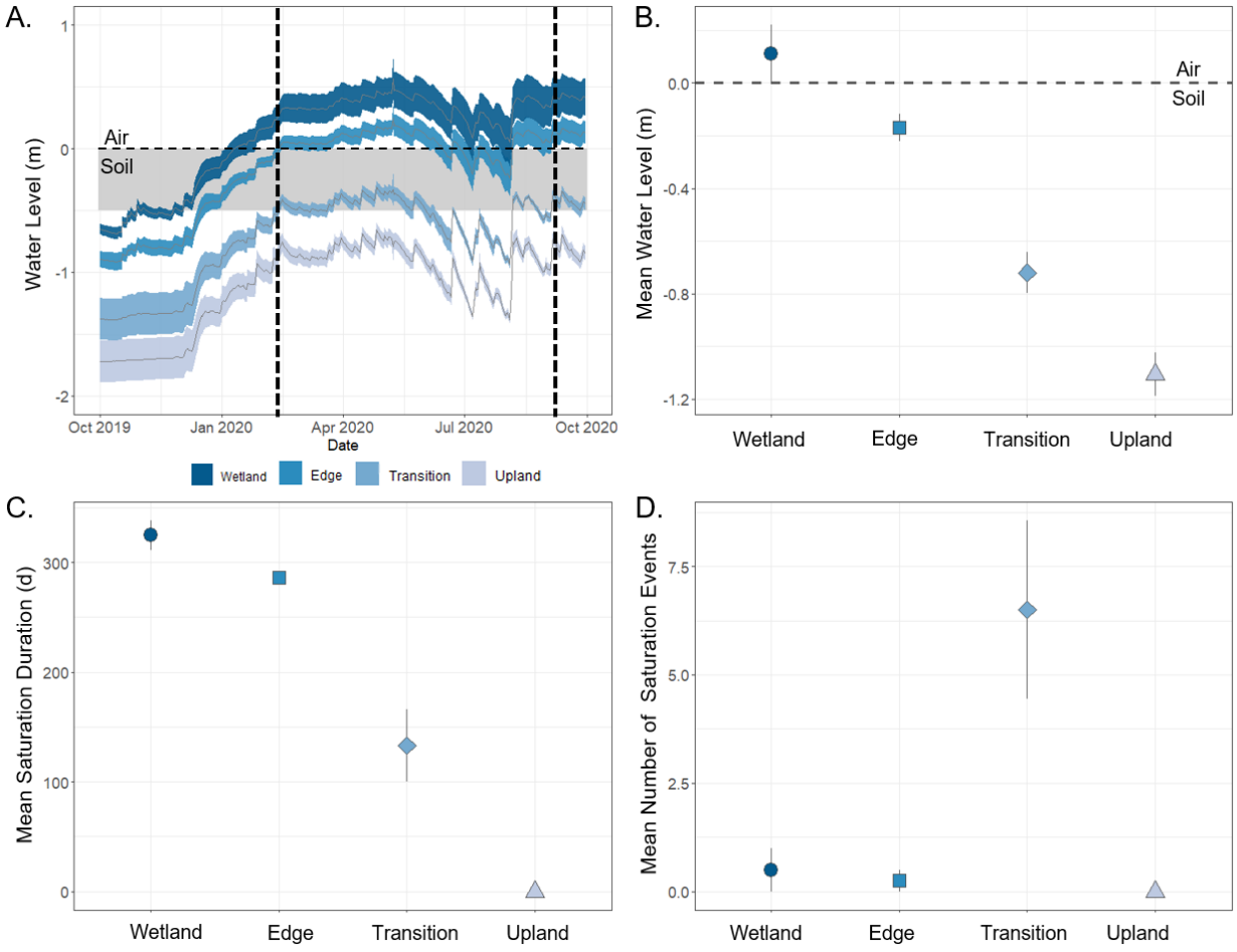

Figure 4.3: Hydrologic metrics averaged across all four sites for the 2020 water year. Ribbon thickness (A) and error bars (B,C,D) represent standard error. (A) Hydrograph of mean water level relative to the ground surface. The gray bar 0 to -0.5 m indicates the soil sampling zone. Vertical dashed lines indicate dates of soil sampling. (B) Mean water level at each transect point relative to ground surface. (C) Mean number of days of saturation above -0.5 m. (D) Mean number of saturation events above -0.5 m.

Table 4.1: Hydrologic characterization of transect points averaged across all four sites during the 2020 water year. Results presented as mean ± standard error and superscripts represent significant differences among transect points using Tukey HSD tests. Water level is relative to the ground surface and negative values indicate water levels below the ground surface.

Transect Point	Elevation Relative to Wetland Center (m)	Minimum Water Level (m)	Mean Water Level (m)	Median Water Level (m)	Maximum Water Level (m)	Mean Saturation Duration (days) Above - 0.5 m	Mean Number of Saturation Events Above - 0.5 m
Wetland	0.46 ± 0.07^{a}	-0.69 ± 0.05^{a}	0.11 ± 0.1^{a}	0.29 ± 0.1^{a}	0.49 ± 0.1^{a}	320 ± 14^{a}	0.5 ± 0.5^{b}
Edge	0.75 ± 0.05^{a}	-0.92 ± 0.07^{a}	-0.17 ± 0.08^{a}	$3.6\text{x}10^{-3} \pm 0.08^{a}$	0.21 ± 0.08^{a}	290 ± 6^{a}	0.25 ± 0.3^{b}
Transition	1.3 ± 0.1^{b}	-1.4 ± 0.2^{b}	-0.72 ± 0.05^{b}	-0.58 ± 0.05^{b}	-0.31 ± 0.05^{b}	130 ± 30^{b}	6.5 ± 2^{a}
Upland	1.7 ± 0.1^{b}	-1.7 ± 0.2^{b}	-1.1 ± 0.06^{c}	-0.98 ± 0.06^{c}	-0.65 ± 0.06^{b}	0 ± 0^{c}	0 ± 0^{b}
Hypothesis Test	ANOVA $F(3,12) = 34.3$ $P < 0.0001$	ANOVA $F(3,12) = 13.3$ $P = 0.0041$	ANOVA $F(3,12) = 43.9$ $P < 0.0001$	ANOVA $F(3,12) = 43.9$ $P < 0.0001$	ANOVA $F(3,12) = 43.9$ $P < 0.0001$	Kruskal-Wallis $X^2=13.5$ $P = 0.004$	Kruskal-Wallis $X^2=11.2$ $P = 0.01$

Table 4.2: Hydrologic characterization of saturation duration and number of saturation events for each soil horizon averaged across all four sites during the 2020 water year. Results presented as mean ± standard error and superscripts represent significant differences among transect points using Tukey HSD tests.

	Mean Saturation Duration (days)			Mean Number of Saturation Events		
Transect Point	**O Horizon**	**A Horizon**	**B Horizon**	**O Horizon**	**A Horizon**	**B Horizon**
Wetland	263 ± 19^{a}	301 ± 23^{a}	307 ± 11^{a}	1.5 ± 1.0	0.25 ± 0.3	1.25 ± 1.0^{b}
Edge	205 ± 32^{a}	269 ± 14^{a}	286 ± 6^{a}	2.5 ± 1.2	1.0 ± 0.6	0.25 ± 0.3^{b}
Transition	0 ± 0^{b}	20.0 ± 20^{b}	136 ± 36^{b}	0 ± 0	1.3 ± 1.3	6.5 ± 1.3^{a}
Upland	0 ± 0^{b}	0 ± 0^{b}	1.0 ± 1.0^{c}	0 ± 0	0 ± 0	0.75 ± 0.8^{b}
Hypothesis Test	ANVOA $F(3,12) = 56.0$ $P < 0.0001$	Kruskal-Wallis $X^2=13.6$ $P = 0.004$	Kruskal-Wallis $X^2=13.4$ $P = 0.004$	ANOVA $F(3,12) = 2.57$ $P = 0.10$	Kruskal-Wallis $X^2=2.53$ $P = 0.47$	Kruskal-Wallis $X^2=9.77$ $P = 0.021$

consistent across both sampling campaigns (Appendix Table A.1). ESOM quantities averaged across all four sites (reported as Extractable Organic Carbon in mg EOC / g soil) decrease from shallow O horizons (0.05 ± 0.008) to deeper B soil horizons (0.01 ± 0.001) regardless of transect point (Table 4.3). EOC generally decreased moving from the Upland to the Wetland (Figure 4.4 Panel A) but was not significantly different (Table 4.4). When EOC is scaled to horizon thickness, the results are relatively consistent across the all transect points and soil horizons, with most values falling between 1 and 7 g EOC/m^2 (Figure 4.4 Panel B).

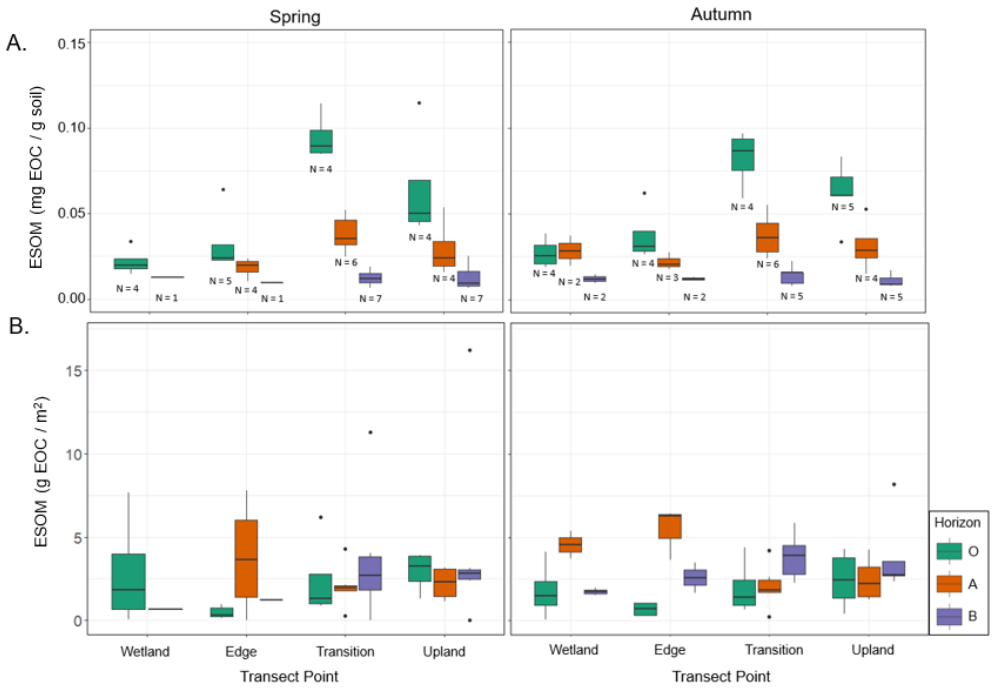

Figure 4.4: ESOM quantities across all four sites from the two sampling campaigns presented by transect point and soil horizon as (A) concentration (mg EOC/g soil) and (B) scaled to soil horizon thickness (g EOC/m^2)

$SUVA_{254}$ values across all four sites generally decreased as soil depth increased. For example, in the spring, O horizons had a mean $SUVA_{254}$ of 2.47 ± 0.1 (L/mg·m) and B horizons had a mean value of 1.35 ± 0.2 (L/mg·m). However, at the Upland and Transition points, $SUVA_{254}$ values were highest in the A horizon (Figure 4.5 Panel A). While $SUVA_{254}$

Table 4.3: ESOM quantity and quality metrics by soil horizon, considering all transect points and all four wetland sites. Results are presented as mean ± standard error and superscripts represent significant differences among transect points using Tukey HSD tests.

	ESOM (log for ANOVA) (mg EOC / g soil)		FI (log for ANOVA)		$SUVA_{254}$ (L/mg·m)		HIX		SSR		Number of samples	
Soil Horizon	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
O	0.052 ± 0.008^{a}	0.053 ± 0.006^{a}	1.49 ± 0.009^{a}	1.50 ± 0.008^{a}	2.47 ± 0.1^{a}	2.27 ± 0.07^{a}	0.526 ± 0.008^{a}	0.504 ± 0.006^{a}	1.25 ± 0.02^{a}	1.39 ± 0.02^{a}	17	17
A	0.030 ± 0.004^{a}	0.031 ± 0.003^{b}	1.52 ± 0.02^{a}	1.51 ± 0.02^{a}	2.48 ± 0.2^{a}	2.70 ± 0.09^{b}	0.526 ± 0.01^{a}	0.531 ± 0.007^{a}	1.34 ± 0.04^{a}	1.30 ± 0.02^{a}	14	15
B	0.012 ± 0.001^{b}	0.013 ± 0.001^{c}	1.68 ± 0.03^{b}	1.67 ± 0.02^{b}	1.35 ± 0.2^{b}	1.59 ± 0.1^{c}	0.455 ± 0.02^{b}	0.456 ± 0.01^{b}	1.54 ± 0.09^{b}	1.99 ± 0.3^{b}	16 (SSR = 13)	14
Hypothesis Test	ANOVA $F(2,44) = 24.9$ $P < 0.0001$	ANOVA $F(2,43) = 38.7$ $P < 0.0001$	ANOVA $F(2,44) = 31.0$ $P < 0.001$	ANOVA $F(2,43) = 46.1$ $P < 0.0001$	ANOVA $F(2,44) = 18.6$ $P < 0.0001$	ANOVA $F(2,43) = 29.7$ $P < 0.0001$	ANOVA $F(2,44)=12.4$ $P < 0.0001$	ANOVA $F(2,43)=5.87$ $P = 0.0019$	ANOVA $F(2,43)=8.10$ $P = 0.0011$	ANOVA $F(2,43)=7.29$ $P = 0.0019$		

Table 4.4: ESOM quantity and quality metrics by transect point, considering all soil horizons and all four wetland sites. Results are presented as mean ± standard error and superscripts represent significant differences among transect points using Tukey HSD tests.

	ESOM (log for ANOVA) (mg EOC / g soil)		FI (log for ANOVA)		$SUVA_{254}$ (L/mg·m)		HIX		SSR		Number of Samples	
Transect Point	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Wetland	0.020 ± 0.004	0.024 ± 0.004	1.52 ± 0.02	1.57 ± 0.03	2.58 ± 0.2	2.16 ± 0.2	0.55 ± 0.005	0.52 ± 0.01^{a}	1.24 ± 0.06	1.30 ± 0.05	5	8
Edge	0.025 ± 0.005	0.027 ± 0.005	1.53 ± 0.03	1.53 ± 0.02	2.38 ± 0.2	2.43 ± 0.1	0.54 ± 0.01	0.52 ± 0.01^{a}	1.29 ± 0.04	1.31 ± 0.04	10	9
Transition	0.041 ± 0.008	0.042 ± 0.008	1.57 ± 0.03	1.56 ± 0.03	2.02 ± 0.2	2.26 ± 0.2	0.50 ± 0.01	0.49 ± 0.01^{a}	1.41 ± 0.05	1.51 ± 0.09	17 (SSR = 15)	15
Upland	0.031 ± 0.007	0.035 ± 0.007	1.60 ± 0.03	1.56 ± 0.03	1.82 ± 0.2	2.02 ± 0.2	0.47 ± 0.02	0.48 ± 0.01^{a}	1.41 ± 0.09	1.86 ± 0.3	15 (SSR = 14)	14
Hypothesis Test	ANOVA $F(3,43) = 0.54$ $P = 0.66$	ANOVA $F(3,42) = 0.67$ $P = 0.58$	Kruskal-Wallis $X^2=1.72$ $P = 0.63$	Kruskal-Wallis $X^2=1.82$ $P = 0.61$	ANOVA $F(3,43) = 1.76$ $P = 0.17$	ANOVA $F(3,42) = 0.96$ $P = 0.42$	ANOVA $F(3,43) = 1.76$ $P = 0.17$	ANOVA $F(3,42) = 2.94$ $P = 0.044$	ANOVA $F(3,42) = 1.23$ $P = 0.31$	Kruskal-Wallis $X^2=10.4$ $P = 0.016$		

values generally increased moving from the Upland to the Wetland, this relationship was not significant (Table 4.4), indicating that soil horizon has a stronger influence on $SUVA_{254}$ values than transect location. FI values increased as soil depth increased where autumn O horizons had a mean FI of 1.5 ± 0.008 and B horizons 1.67 ± 0.02 (Figure 4.5 Panel B). Similar to $SUVA_{254}$, soil horizon is the stronger control on FI values. HIX values decreased with soil depth where in autumn, mean O horizon HIX was 0.50 ± 0.006 while mean B horizon HIX was 0.46 ± 0.01. Maximum HIX values frequently occur in the A horizon along the transect (Figure 4.5 Panel C), where the mean A horizon HIX was 0.53 ± 0.007 in autumn. HIX values increase moving from the Upland to the Wetland, and this relationship was significant in autumn (Table 4.4). Mean SSR values of ESOM decreased from Upland to Wetland and from O to B soil horizons. The Transition and Upland B horizon SSR had a larger range of values compared to all other soil horizons (Figure 4.5 Panel D). When assessing the interaction of both soil horizon and transect location on ESOM quantity and quality, the ANCOVA resulted in few significant differences.

The ESOM fluorescence metrics show patterns when compared to each other. For example, as FI values increase, indicating more microbial-like organic matter, HIX values decrease, indicating less ring structure within the organic matter (Figure 4.6 Panel A). Similarly, as FI increases, $SUVA_{254}$ values decrease, indicating less aromatic organic matter is present in ESOM (Figure 4.6 Panel B). The k-means cluster analysis demonstrated that O and A horizons are grouped together while B horizons are distinctly separate in ESOM composition characteristics. Soil horizons with higher ESOM quantities have higher $SUVA_{254}$ and lower FI values (Appendix Figure A.5). Additionally, ESOM composition results fall within the continuum of organic matter sources at the wetland sites. For example, ESOM from B horizons had similar FI values to upland groundwater wells, while O and A horizons had FI values similar to wetland surface water wells and extracted leaf litter (Figure 4.7).

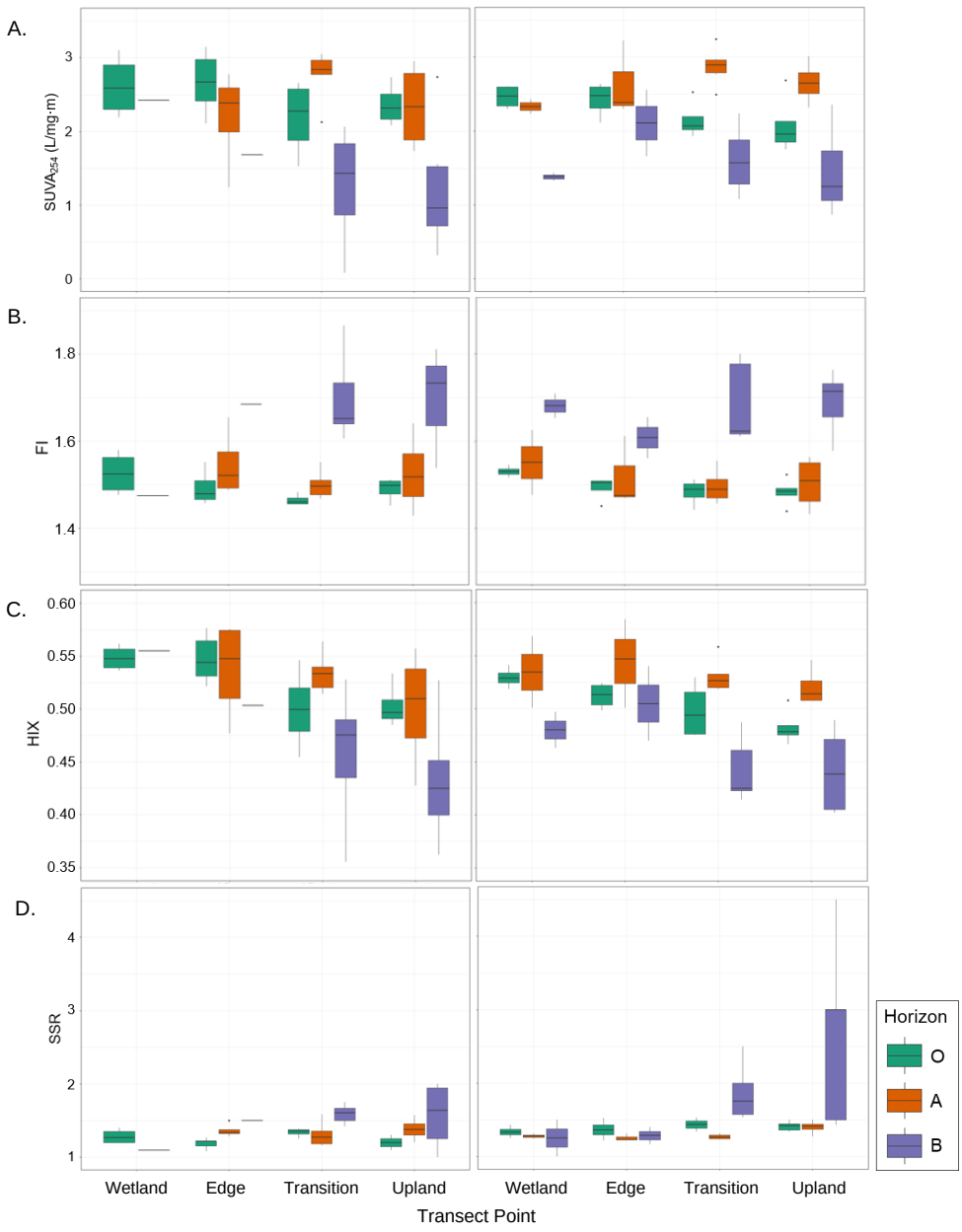

Figure 4.5: ESOM quality results across the four sites from the two sampling campaigns presented by transect point and soil horizon: (A) Specific UV Absorbance at 254 nm ($SUVA_{254}$), (B) Fluorescence Index (FI), (C) Humification Index (HIX), and (D) Spectral Slope Ratio (SSR).

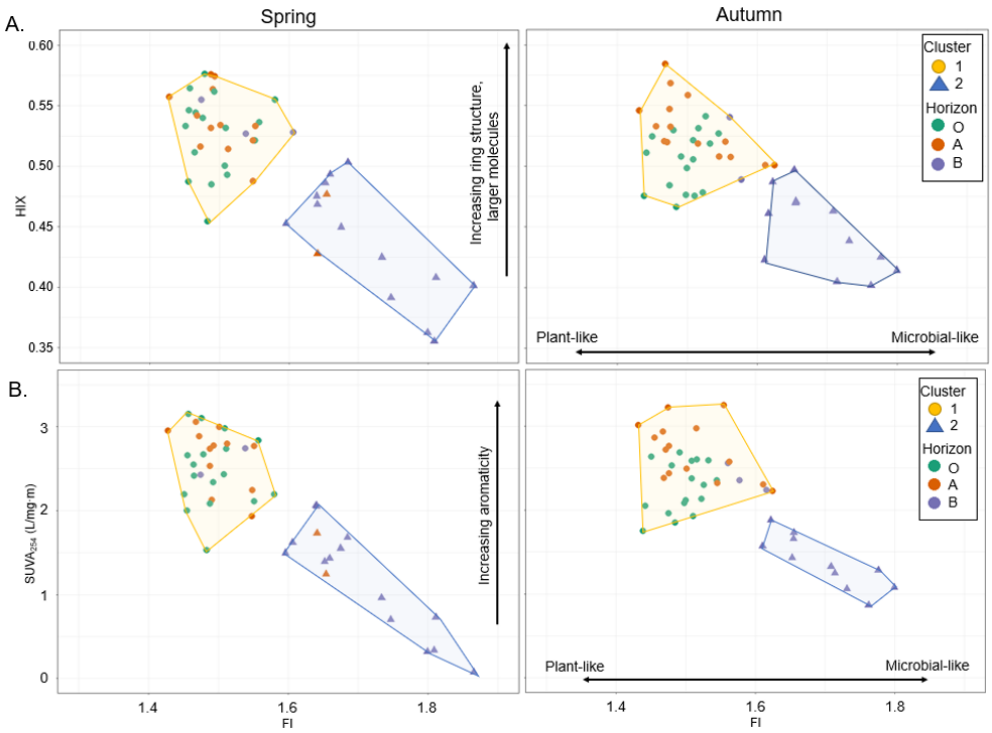

Figure 4.6: (A) HIX vs FI and (B) $SUVA_{254}$ vs FI over the two sampling campaigns. Shape indicates k-means cluster grouping and color indicates soil horizon.

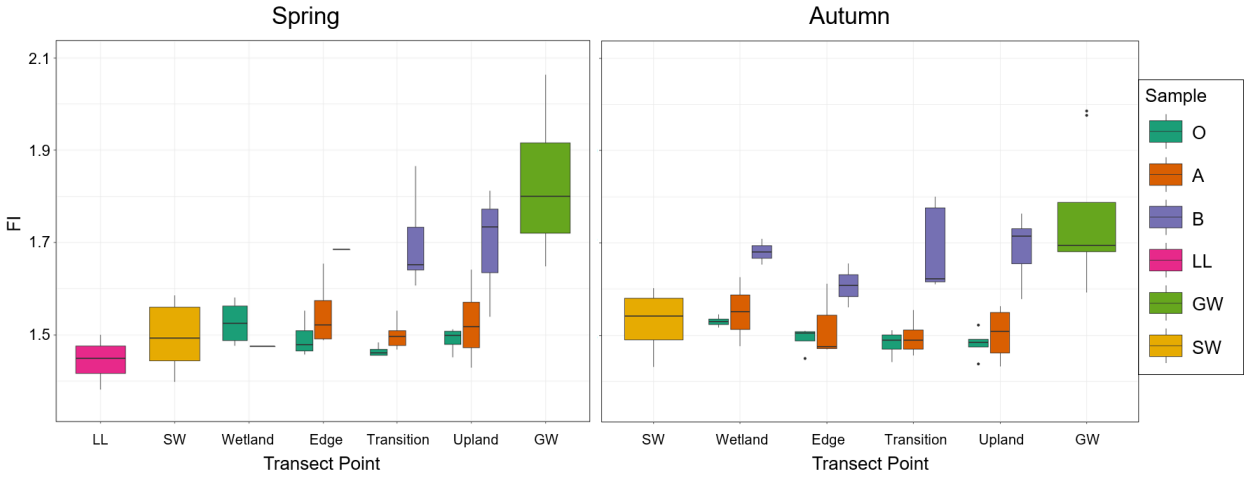

Figure 4.7: ESOM composition (FI) results compared to leaf litter (LL) extracts, groundwater (GW), and surface water (SW) samples across the four wetland sites.

4.4 PARAFAC Modeling

To further explore the composition of ESOM, EEMs were collected and analyzed by applying the Cory and McKnight (CM) PARAFAC model (Cory and McKnight, 2005). Across both sampling campaigns, all transect points, and all soil horizons, Components 2 (C2) , C4, and C12 of the CM model had the highest percent loadings (Figure 4.8). C2 and C12 have been characterized by high molecular weights where C2 is likely terrestrial or SOM derived, and C12 likely results from autochthonous production. C4 has been correlated with acetal and ketal carbon and may be sourced from terrestrial organic matter or microbially processed organic matter (Appendix Table A.3). C2 and C4 typically had highest loadings in O and A horizons regardless of transect point (Figure 4.9). Components C8 and C13 have amino acid signatures, where C8 is Tryptophan-like and indicative of less degraded peptide material, and C13 is Tyrosine-like, indicative of more degraded peptide material. Despite having relatively low percent loadings across the samples, C8 is more prominent in B horizons, while C13 is highest in O horizons and decreases down the soil profile (Figure 4.9). B horizons along the transect have the largest ranges in component loadings. Percent loadings of the CM model showed trends with the other fluorescence metrics calculated. For example, as $SUVA_{254}$ increases, percent C2 increases (Figure 4.10 Panel A), and percent protein, the sum of C8 and C13, is more prominent in soils with higher FI values (Figure 4.10 Panel B).

A Delmarva specific PARAFAC model was also applied to the ESOM data. Components 1 and 2 of the Delmarva Synoptic PARAFAC model had slightly elevated loadings across both sampling campaigns compared to components 3 and 4 (Appendix Figure A.6). Previous studies have identified components 1 and 2 as terrestrially sourced; whereas, components 3 and 4 are likely microbially-sourced (Appendix Table A.5). There are no distinct

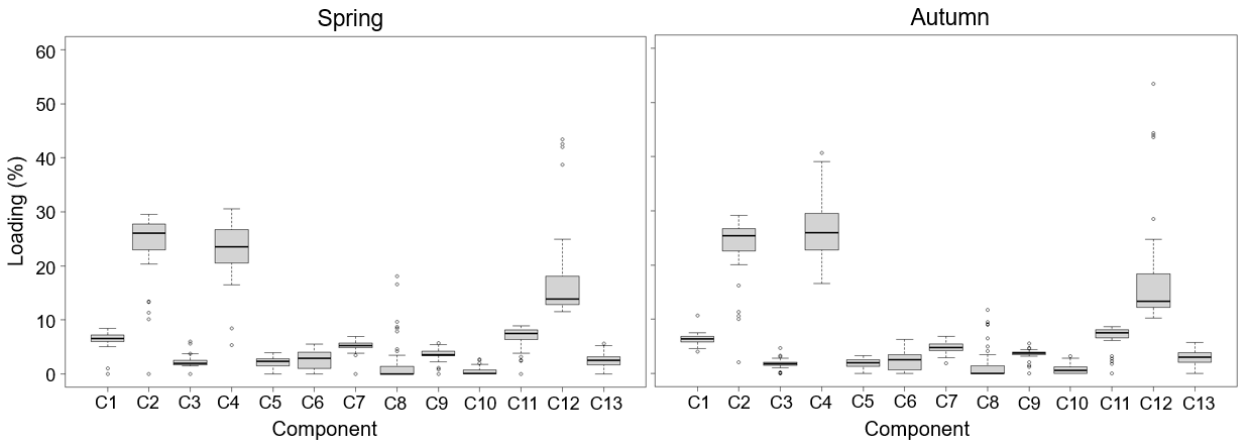

Figure 4.8: ESOM sample loadings for the 13 component Cory and McKnight PARAFAC model across all wetland sites during the two sampling campaigns.

patterns in sampling loadings along the transect or within soil horizons for each of the four components nor do the loadings demonstrate trends with other fluorescence metrics (Appendix Figure A.7).

4.5 Between Site Variations

When analyzing hydrologic metrics, wetland morphology, soil characteristics, and ESOM results, it became apparent that there were two distinct groupings within the four wetlands sampled. Soil color differences in B horizons at the Transition point were the first indicator of these between-site differences; therefore, the following comparison was performed based on Transition point results. Wetland 1 (ND) and Wetland 2 (DB) had shorter transects (19 and 23 m) and increased slopes (∼0.07) between the wetland monitoring well and the Transition location (Table 4.5). These wetlands had lower minimum, mean, and maximum water levels, indicating that the sites were drier during the 2020 water year. Transition soil horizons in Wetlands 1 and 2 had similar coloration to the Upland location. Whereas, Wetland 3 (TB) and Wetland 4 (QB) had longer transects (35 and 41 m) with lower slopes

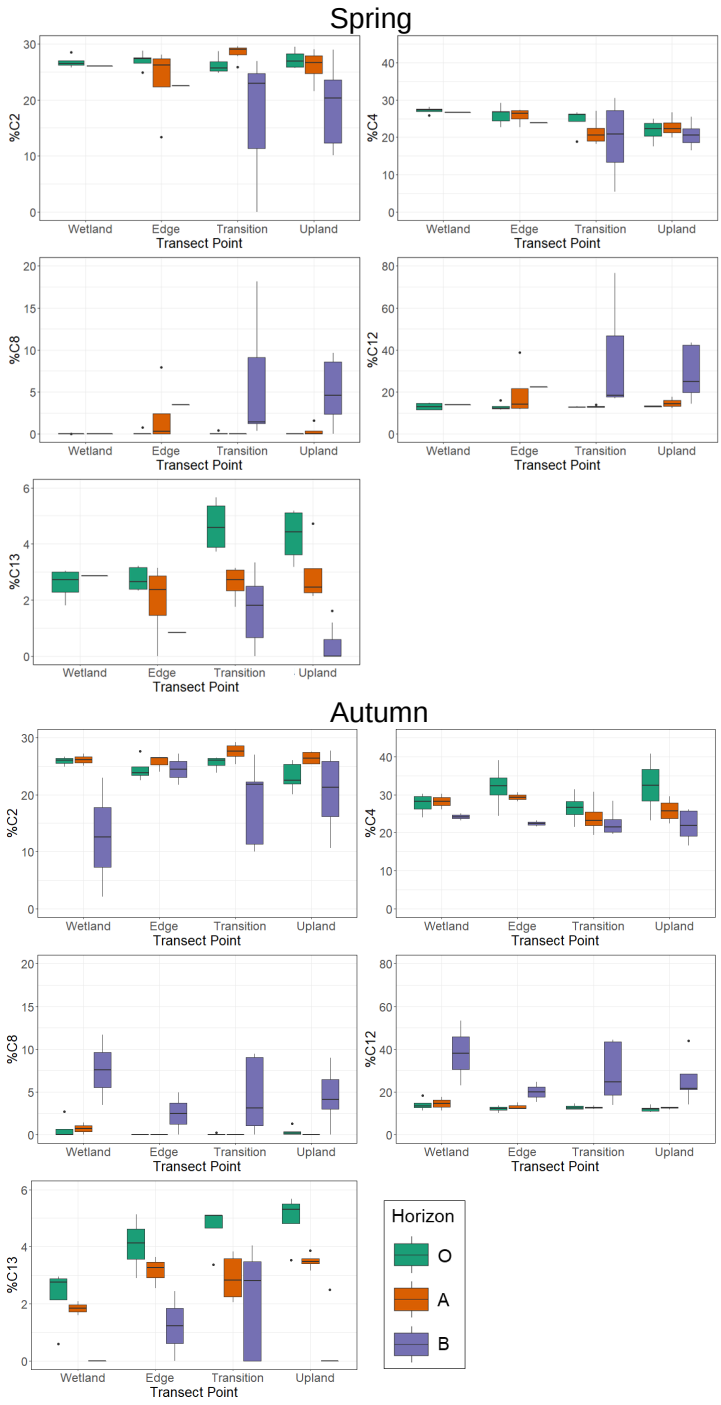

Figure 4.9: Percent loading of notable components from the Cory and McKnight PARAFAC model within each soil horizon at each transect point across the four sites.

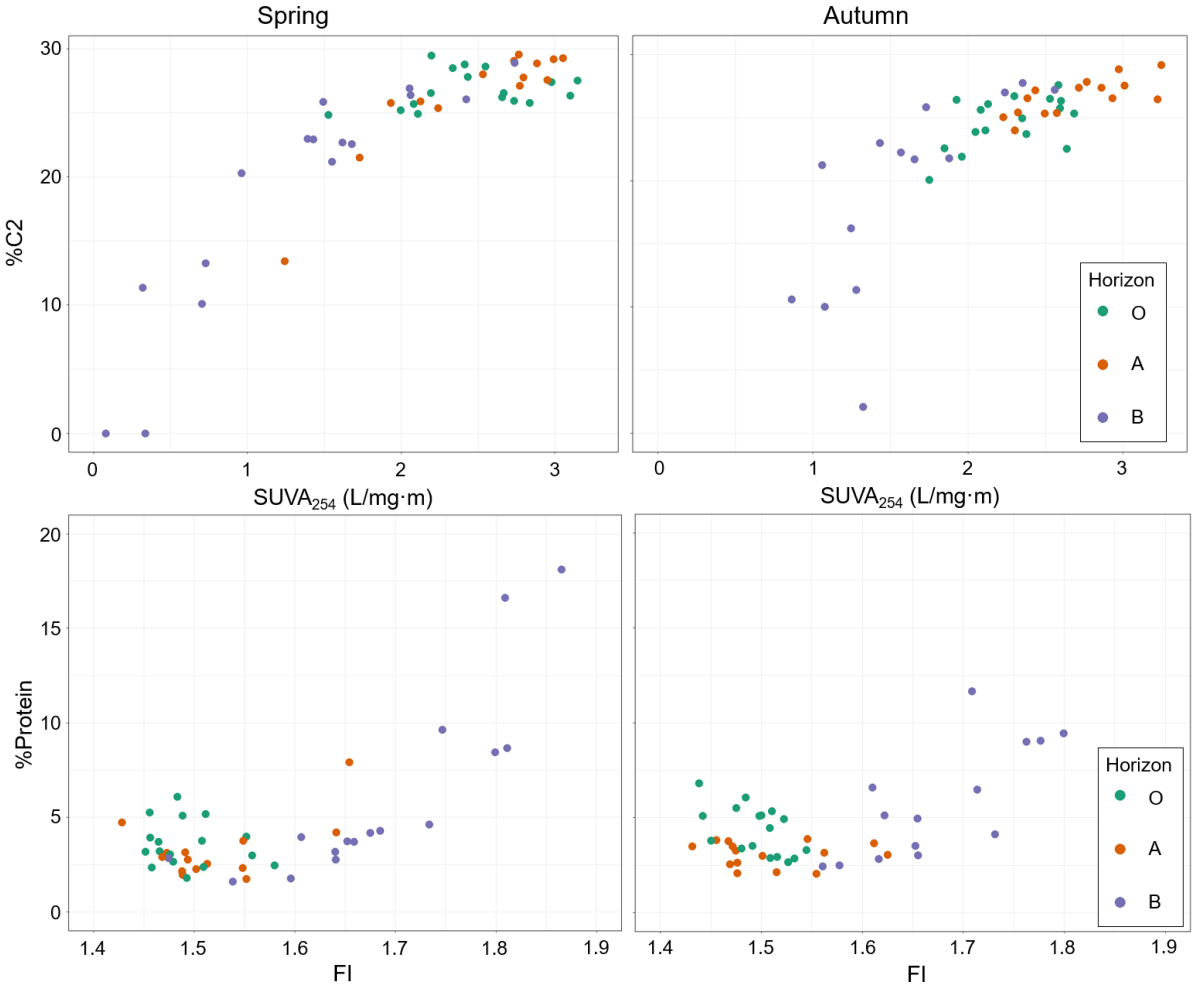

Figure 4.10: Percent loadings from the Cory and McKnight PARAFAC model of (A) Component 2 vs $SUVA_{254}$ and (B) Protein (C8 + C13) vs FI. Values are colored by soil horizon.

(∼0.03). This resulted in higher minimum, mean, and maximum water elevations, relative to the ground surface, at the Transition point. The wetter conditions at these two sites cause Transition soils to have a darker matrix that more closely resembled the Wetland and Edge soils. All four wetlands had similar water level coefficients of variation and number of saturation events in the B horizon (Table 4.5). Notably, the duration of saturation is significantly lower in Wetland 3, but this is due to the depth of sample reaching approximately 10 cm less than the other sites. If samples had extended to a depth of 50 cm, the B horizon would've been saturated for 151 days, making it comparable to the other three wetland sites. These hydrological and morphological site differences are likely influencing ESOM composition. Although ESOM fluorescence metrics were within a similar range of values for all four sites, the drier wetlands (1 and 2) have a larger spread in fluorescence metric results. This was particularly true for Transition and Upland B horizons (Figure 4.11, 4.12, Appendix Figures A.9, A.10, and A.11).

Table 4.5: Individual site characteristics and hydrologic metrics at the Transition transect location.

Transition Point Characteristics	Wetland 1 (ND)	Wetland 2 (DB)	Wetland 3 (TB)	Wetland 4 (QB)
Elevation relative to wetland well (m)	1.5	1.4	1.2	0.97
Distance from wetland well (m)	23	19	41	35
Slope (m/m)	0.068	0.074	0.030	0.028
Minimum water level (m)	-1.8	-1.4	-1.3	-1.0
Mean water level (m)	-0.91	-0.74	-0.69	-0.54
Maximum water level (m)	-0.42	-0.35	-0.31	-0.17
Water level coefficient of variation	52	48	45	50
B Horizon top elevation (m)	-0.14	-0.19	-0.13	-0.30
B Horizon bottom elevation (m)	-0.64	-0.56	-0.38	-0.45
Duration of saturation in the B horizon (days)	166	170	30	180
Number of saturation events in the B horizon	6	6	10	4

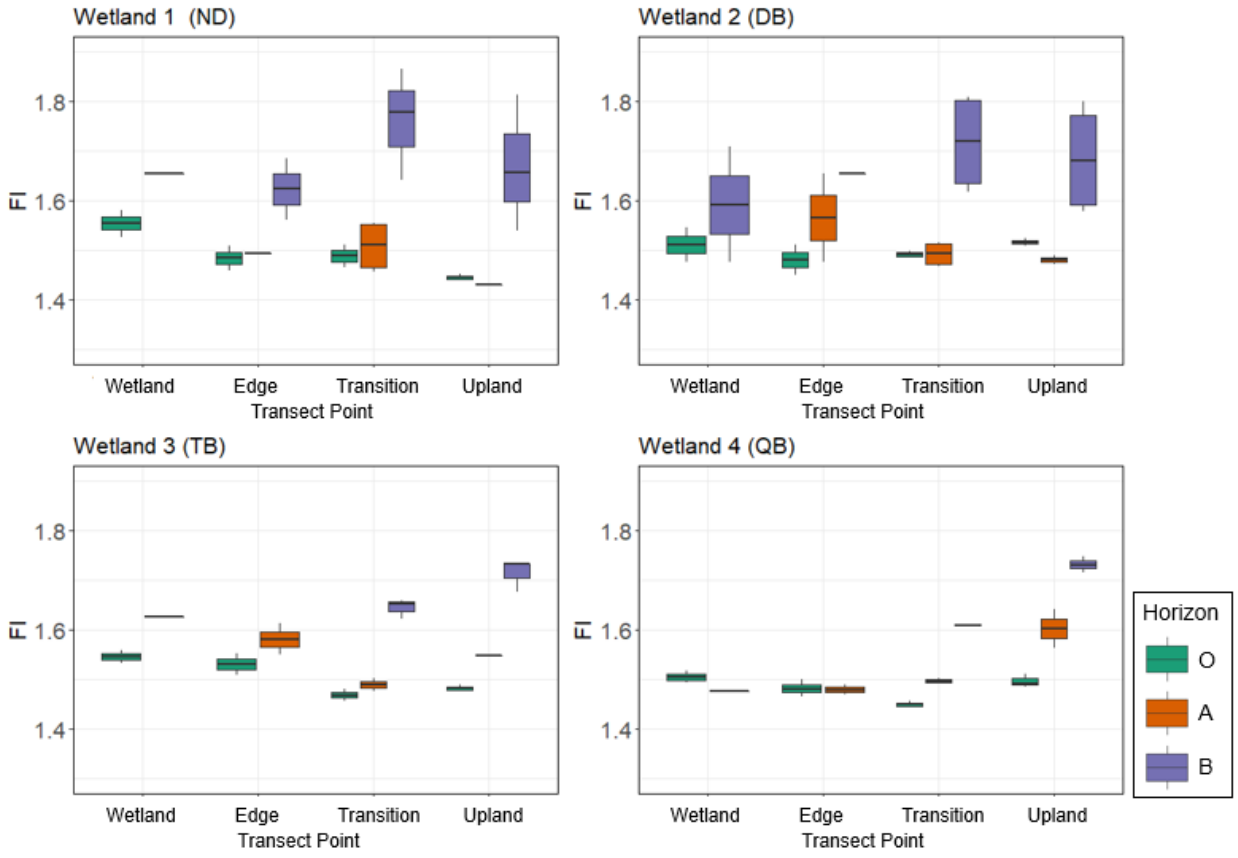

Figure 4.11: FI values across the transect and soil horizons for both sampling campaigns separated by wetland site.

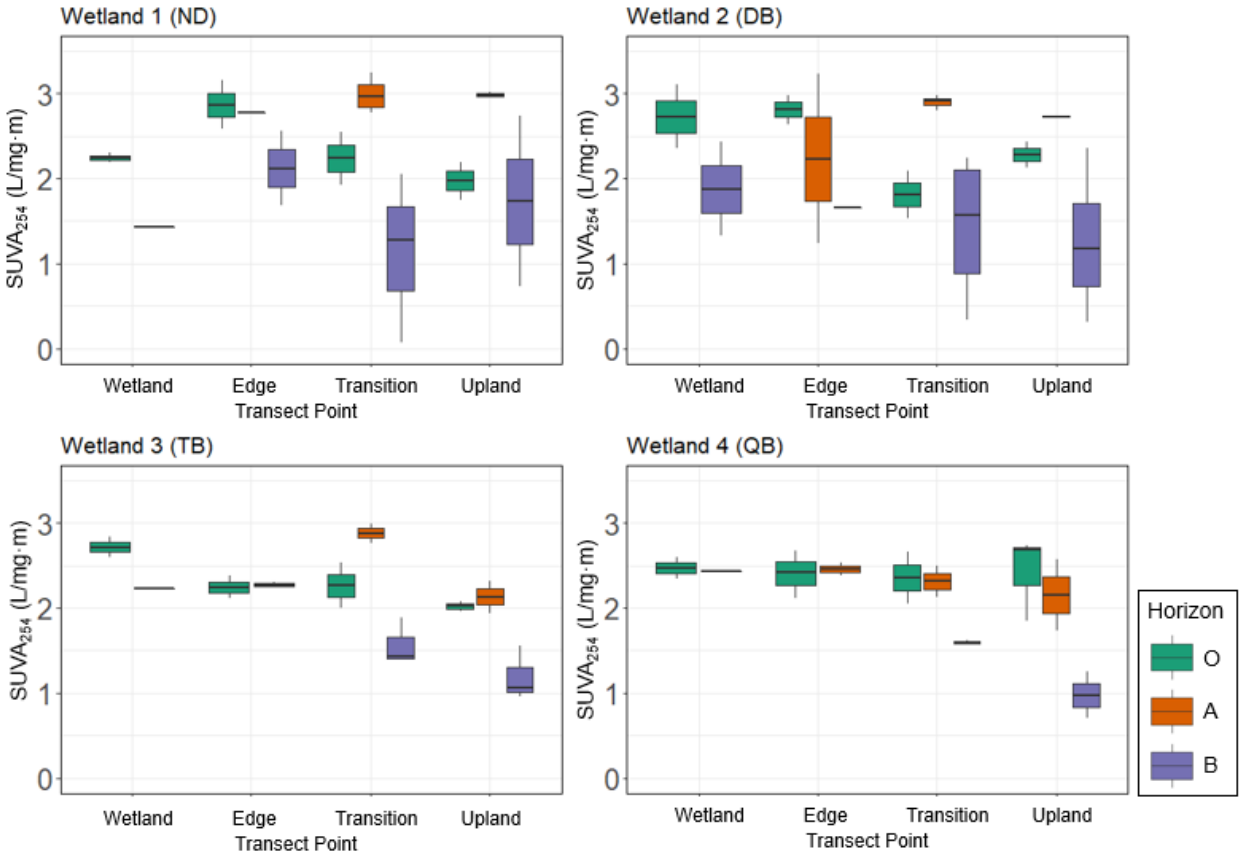

Figure 4.12: $SUVA_{254}$ values across the transect and soil horizons for both sampling campaigns separated by wetland site.

4.6 Potential DOM Release

ESOM quantities measured at the time of sampling represent the pool of organic matter that has the potential to be mobilized into DOM. For example, across all four sites, Upland O horizons were thin but contained a large quantity of ESOM, while Wetland O horizons were thick but had less ESOM. By scaling ESOM quantity to horizon thickness, the potentially mobile pool of DOM was larger for the Upland O horizons at 3.2 g C/m^2 compared to Wetland O horizons at 1.4 g C/m^2 (Table 4.6). Considering how saturation duration of these soil horizons influences the DOM release that will occur, the Upland O horizons were never saturated across all four sites, leading to a constant potential with zero anticipated release. In contrast, the Wetland O horizons were saturated for a mean of 68 days from the start of the 2020 water year to the time of spring soil sampling, indicating that a certain proportion of ESOM was already released to DOM in Wetland O horizons at the time of sampling (Figure 4.13). Utilizing a first order decay function, the theoretical starting potential of the Wetland O horizons was calculated to be 2.7 g EOC/ m^2. Starting from this initial potential of 2.7 g EOC/ m^2, Wetland O horizons potentially contributed 1.3 g EOC/m^2 during the wetting up period of the 2020 water year (Table 4.6).

Table 4.6: Potential DOM release results for the Wetland and Upland O horizons after applying the first order decay model, where C_0 is the estimated initial ESOM at the start of the 2020 water year and C_{sample} is ESOM quantity at the time of spring sampling. Mean bulk densities from Kottkamp (2019), O horizon thickness, saturation duration from October 1, 2019 to March 10, 2020, and resulting predicted ESOM release during this saturation period are also presented.

		Mean Bulk Density*	Mean Horizon Thickness	Saturation Duration	C_0 (Prior to 2020 WY saturation)	C_{sample} (At time of spring sampling)	ESOM Released
Location	**Horizon**	g / cm^3	cm	d	g EOC / m^2		g EOC / m^2
Wetland	O	0.33	18.4	68	2.7	1.4	1.3
Upland	O	0.63	7.8	0	3.2	3.2	0

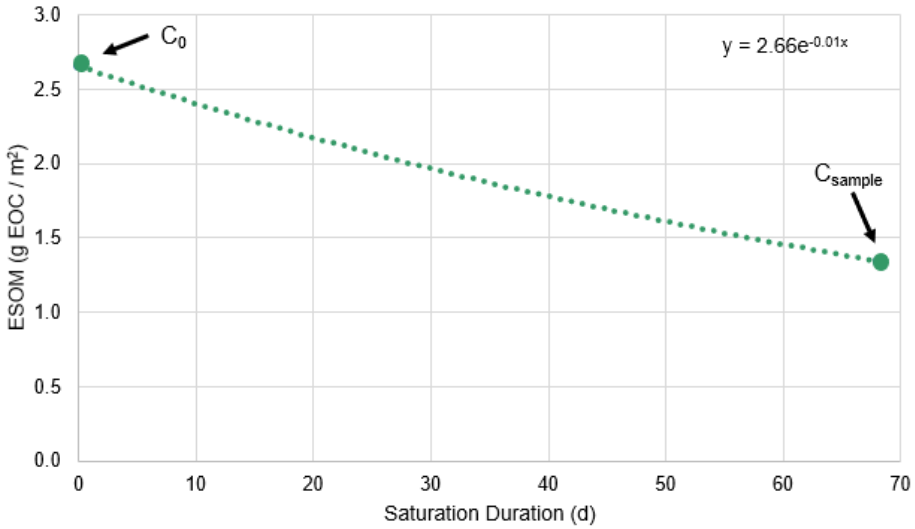

Figure 4.13: Estimated ESOM export from the Wetland O horizon using the first order decay model.

Chapter 5

Discussion

5.1 ESOM quantity and quality is controlled by transect position and soil horizon

The landscape position of each transect point results in distinct hydrologic regimes that vary from unsaturated for the majority of the year to saturated nearly continuously. These distinct hydrologic regimes influence resulting plant growth, microbial activity, and soil development at each transect point. For example, soils at the Wetland location are saturated 72% of a typical water year, leading to the development of hydric soil features, such as dark and depleted matrix colors. The Wetland locations have little to no aquatic vegetation present and the constant saturation favors methanogen microbial communities, methane emissions, and macroaggregate carbon stabilization (Hondula et al., 2021, Kottkamp, 2019, Maietta et al., 2020). In contrast, the Upland position was never saturated in the upper 50 cm of the soil profile by the groundwater table in the 2020 water year and therefore only received moisture via throughfall that infiltrated into the soil profile. This causes the Upland soil environment to be favorable to aerobic microbial communities, there are reduced methane emissions, and organic matter is protected, or less accessible to microbial degradation, via organo-mineral associations (Hondula et al., 2021, Kottkamp, 2019, Maietta et al., 2020). These landscape differences along the upland to wetland transect

influence net processes acting on SOM and the resulting ESOM quantity and quality. There were non-significant trends of decreasing ESOM moving from the Upland to the Wetland because saturated wetland conditions cause soil DOM release to have occurred prior to ESOM extractions. When ESOM quantities are scaled to horizon thickness, results are relatively consistent across the transect. This is because shallow Upland horizons are thin with a high concentration of ESOM, while shallow Wetland soils have low ESOM concentrations but increased horizon thickness. There were non-significant trends of increasing $SUVA_{254}$ and HIX values moving from the Upland to the Wetland reflecting the influence of saturated conditions slowing down organic matter processing, allowing ESOM to remain more aromatic and complex. Between-site variations demonstrated that hydrologic regime and wetland morphology influence ESOM composition, particularly in the B horizons of Transition and Upland soils. Sites with lower mean water levels had more variability in FI, $SUVA_{254}$, and HIX values, whereas the opposite was true for sites with higher mean water levels. Sites with higher mean water levels likely have consistent low redox conditions that constrain microbial degradation processes, resulting in less variability in ESOM quality.

Despite landscape positioning creating distinct hydrologic regimes along the transect, soil horizon tended to be a stronger control on ESOM composition than transect location. The sorption-desorption and microbial degradation processes that occur as organic matter cycles down the soil profile lead to a transition from plant-like to microbial-like signatures in DOM (Kaiser and Kalbitz, 2012, Shen et al., 2015). For example, fresh plant-derived organic matter inputs are more effectively adsorbed to the mineral matrix (Kaiser and Guggenberger, 2000, Roth et al., 2019), leading to exchange with older or partially microbially altered organic matter, which then migrates to deeper soil horizons (Kaiser and Kalbitz, 2012, Müller et al., 2009). In this study, ESOM from shallow soil horizons is more plant-like, aromatic, and has more ring structure, as indicated by lowest FI, highest $SUVA_{254}$, and highest HIX

values occurring in O and A horizons. This is expected because the O and A horizons are the first to receive leaf litter and other plant biomass inputs. HIX and $SUVA_{254}$ reached maximum values in A horizons, suggesting A horizons have a large capacity for sorption of aromatic organic matter, potentially as a result of organo-mineral interactions common in Delmarva upland mineral soils (Kottkamp, 2019). In contrast, FI increases while $SUVA_{254}$ and HIX both decrease in B horizons indicating that ESOM becomes more microbial-like, less aromatic, and has less ring structure deeper in the soil profile after a combination of physical, chemical, and biological processes alter the organic matter composition. B horizons tended to have the most variability in ESOM results. Fluctuations between saturated and unsaturated conditions as the groundwater table rises and falls likely promotes a more diverse range of biogeochemical processes acting on SOM in B horizons (D'Amore et al., 2010, Marton et al., 2015). For example, soil-DOM mobilization studies have found that alternations between saturated and unsaturated conditions promote SOM mineralization and influence reactions on mineral surfaces that act to release or adsorb organic matter (Reddy and Patrick, 1975, Rouwane et al., 2018).

The fluorescence metrics calculated using ESOM optical data fall within the ranges reported by other ESOM, wetland soil, and whole water DOM studies (Appendix Table A.2). Two previously created PARAFAC models were employed to decompose the fluorescence signature of ESOM samples. It was initially surprising that the Cory and McKnight model, which was developed with a large range of end member samples, performed better than the Delmarva Synoptic model, which was developed from surface water and groundwater samples from 20 Delmarva GIWs during the 2020 water year. The Cory and McKnight model results also held up with other trends in ESOM metrics. Lack of trends with ESOM metrics and the presence of significant patterns in Delmarva Synoptic model residuals suggest that the Delmarva Synoptic PARAFAC model isn't capturing all the variation in ESOM data. In

this case, the large range of end members in the Cory and McKnight model make the model more likely to capture all the fluorescence in the ESOM data. These results suggest the Delmarva Synoptic model would benefit from an increased number of end-members (e.g. ESOM, leaf litter extracts) being utilized to create a PARAFAC model for the Delmarva Bay GIW system.

5.2 Relative contributions of soil-derived DOM along upland to wetland transects

It is well known that saturated conditions in wetland soils slow down decomposition rates allowing organic matter to accumulate (Catalán et al., 2016, Kayranli et al., 2010, Villa and Bernal, 2018). This led to the expectation that Wetland and Edge soils would have higher amounts of organic matter available for mobilization. However, ESOM was lowest in the Wetland and Edge soil horizons because these soil horizons are saturated almost constantly during a typical water year. Saturation creates anaerobic soil conditions promoting iron and manganese solubilization, favoring DOM release (Grybos et al., 2009). Therefore, any potentially mobile SOM has been mobilized, leaving behind less ESOM compared to Transition and Upland soil horizons. The conceptual first order decay function demonstrated that Wetland O horizons initially had pools of potentially mobile organic matter similar to that of the Upland O horizons, reconciling the discrepancy between wetland SOM accumulation and ESOM results. Simply, the wetland soil DOM export has been realized by the nearly constant saturation of the Wetland horizons. In contrast, the Upland O horizons were never saturated by the groundwater table in the 2020 water year, leading to any potential export remaining largely unrealized. The conceptual DOM release model demonstrated that while ESOM is relatively low in the Wetland soils, these soils still remain the most significant

for potential DOM export due to their hydrologic connectivity in the Delmarva Bay GIW system. These results also indicate that the composition of ESOM from Wetland and Edge soils best represents the organic matter left behind after flushing of the soil profile; whereas, ESOM composition from the Upland and Transition likely represents organic matter that has yet to be mobilized. Kottkamp (2019) reported SOM stocks ranging from 4,000 to 12,000 g C/m^2 in shallow Delmarva Bay wetland soil horizons. This indicates that ESOM, which typically ranged from 1 to 7 g EOC/m^2, represents an extremely small proportion of Delmarva Bay SOM stocks. Despite making up such a small percentage of SOM stocks, ESOM released from wetland soils may fuel the anaerobic microbial communities found in Delmarva Bay wetland soils (Maietta et al., 2020) leading to greenhouse gas emissions (Hondula et al., 2021).

Comparing ESOM to groundwater and surface water composition results further confirmed that Delmarva Bay surface water DOM composition likely reflects organic matter inputs from shallow soil horizons, in addition to any direct plant biomass inputs or aquatic microbial activity. Because Edge and Wetland O and A horizons are continuously in contact with the inundated footprint of the Delmarva Bays, wetland surface water was most similar to ESOM from O and A horizons while groundwater was most similar to ESOM from B horizons. Any additional soil-derived DOM inputs would likely be most prominent during times where the wetland footprint expands and the aquatic-terrestrial interface shifts to soils that had previously been unsaturated. Previous studies have shown that DOM release is greatest during the transition from oxic to reducing conditions (Rouwane et al., 2018). This suggests that the Transition transect zone is important because it experiences the largest number of fluctuations between saturated and unsaturated conditions. Because O and A horizons are rarely saturated in the Upland and Transition, B horizons are expected to be the largest contributors of soil-derived DOM from these locations. ESOM quality metrics demonstrated

that DOM exported from B horizons will be less aromatic, have lower molecular weight, and have a microbial-like signature, reflecting a composition that has been associated with labile DOM (Balcarczyk et al., 2009, Fellman et al., 2008). However, the surface water chemistry is distinctly different from B horizon ESOM and groundwater DOM composition, suggesting that any signal from groundwater inputs is being overshadowed by the surface water and organic soil horizon inputs. Alternatively, when B horizon derived DOM arrives in the wetland, it may be rapidly mineralized by aquatic microorganisms, resulting in the low bioavailability of DOM previously observed in Delmarva Bay surface waters (Hosen et al., 2018). If the wetland is losing surface water to groundwater during dry periods, the signature of DOM exported will likely be altered during transport through the soil by biological (microbial mineralization) and physical (sorption to mineral surfaces) processes.

5.3 Limitations and Future Work

It is important to note that ESOM represents a conservative estimate of the pool of SOM that may be mobilized into DOM. However, it may overestimate the amount of DOM mobilized because lab experiments may cause cell lysis and the extraction procedure destroys the natural soil structure, exposing organic matter that would otherwise remain trapped in soil pores (Rennert et al., 2007). Although calcium chloride is considered a conservative extractant solution by previous ESOM experiments (Cincotta et al., 2019, Rennert et al., 2007), this solution has a different composition than rainwater, surface water, or groundwater found at the Delmarva sites, which will impact the amount of organic matter extracted.

ESOM quantities did not change significantly between the spring and autumn sampling events, indicating there are organic matter accumulation mechanisms replenishing the potentially mobile pool of SOM in Delmarva Bay soils which were not taken into account by

the first-order decay model. For example, there are fresh organic matter inputs to the soil system each year when leaf-off occurs, and as plant biomass decomposes it becomes incorporated into SOM. Additionally, soil development processes and SOM accumulation occur on timescales of hundreds of years, leading to uncertainty in whether significant changes to the ESOM stock would be detected over just one year of sampling. Because the first order decay equation was applied based on laboratory DOM release studies of non-wetland soils, the equation may overestimate the true rate of organic matter release from soils. This study estimated DOM release from the continuously dry (Upland) and continuously saturated (Wetland) landscape positions over one saturation event by focusing on the period of wetting up over the winter. Future DOM release modeling along upland to wetland transects should consider how fluctuations between saturated and unsaturated soil conditions influence DOM release.

Although soil horizon was the stronger control on ESOM quality, there are likely differences between Upland and Wetland ESOM that fluorescence metrics alone cannot detect. For example, determining the age of ESOM at the different transect locations using carbon isotopes could distinguish the stage of decomposition and how long the organic matter has been part of GIW carbon cycling processes. Bioavailability experiments could be performed using ESOM to explore if soil-derived DOM would be rapidly processed or mineralized by soil and aquatic microbes. Inundation at the Wetland and Edge points often made it difficult to get samples as deep as Transition and Upland locations; therefore, Wetland and Edge B horizon results would benefit from sampling to increased depths. Because soils were sampled during wet periods, future work could explore how ESOM quantity and quality would change if soils were sampled during prolonged dry periods at these wetland sites. Additionally, this study only considered saturation events from the groundwater table, which ignores rainfall infiltration as a saturation source that can mobilize DOM. Further work is

needed to quantify the amount, composition, and fate of DOM released from soils along the aquatic-terrestrial interface as a result of seasonal groundwater table fluctuations and infiltration during precipitation events.

Chapter 6

Conclusions

Previous studies have shown the importance of DOM in freshwater systems (e.g. food webs, drinking water). Therefore, despite ESOM representing only a small pool of potentially mobile organic matter relative to SOM stocks, DOM mobilization from wetland soils has implications for aquatic biota, human health, and downstream water quality. This study was the first to use the ESOM procedure along a hydrologic gradient from upland to wetland. These results were also the first to confirm that wetland soil ESOM follows the typical organic matter concentration and composition trends found in other soil systems (e.g. decreasing organic matter concentration and increasing microbial signatures with increasing soil depth).

Carbon cycling in Delmarva Bays is fundamentally controlled by the hydrologic regime's influence on landscape processes such as soil development, microbial communities, and plant biomass on long-term time scales. The thick wetland soils are most significant as potential sources of DOM despite having low quantities of ESOM relative to upland soils because they are hydrologically connected to the wetland surface water through the majority of the water year (Figure 6.1). Soils at the aquatic-terrestrial interface, such as B horizons at the Transition location, are expected to play an important role in carbon cycling because they experience a higher number of fluctuations between saturated and unsaturated conditions. Shallow Upland and Transition soils store a lot of ESOM in their thin horizons, serving as a carbon storage location on the GIW landscape, but lack of hydrologic connectivity limits

the movement of this organic matter.

As climate change is expected to alter the hydrologic regime through increasingly intense precipitation events, higher evapotranspiration demands in the summer, and warmer global temperatures, the aquatic-terrestrial interface is likely to shift. This could lead to previously unsaturated soil horizons, such as in the Transition zone, becoming DOM sources to the Delmarva Bay landscape. Saturation of historically less hydrologically connected soils could breakdown organo-mineral associations, causing soils that are normally a carbon sink to become a carbon source. By analyzing the potentially-mobile pool of DOM, this study adds to previous research that has explored soil carbon stabilization mechanisms, DOM composition of surface waters, and greenhouse gas emissions within the Delmarva Bay GIW system. Although restored wetlands have lower SOM stocks compared to natural wetlands (Fenstermacher et al., 2016) and it takes many years to redevelop natural soil properties (Wolf et al., 2011), this work provides insight to future potential DOM sources as restoration continues in Delmarva Bays and other wetland systems.

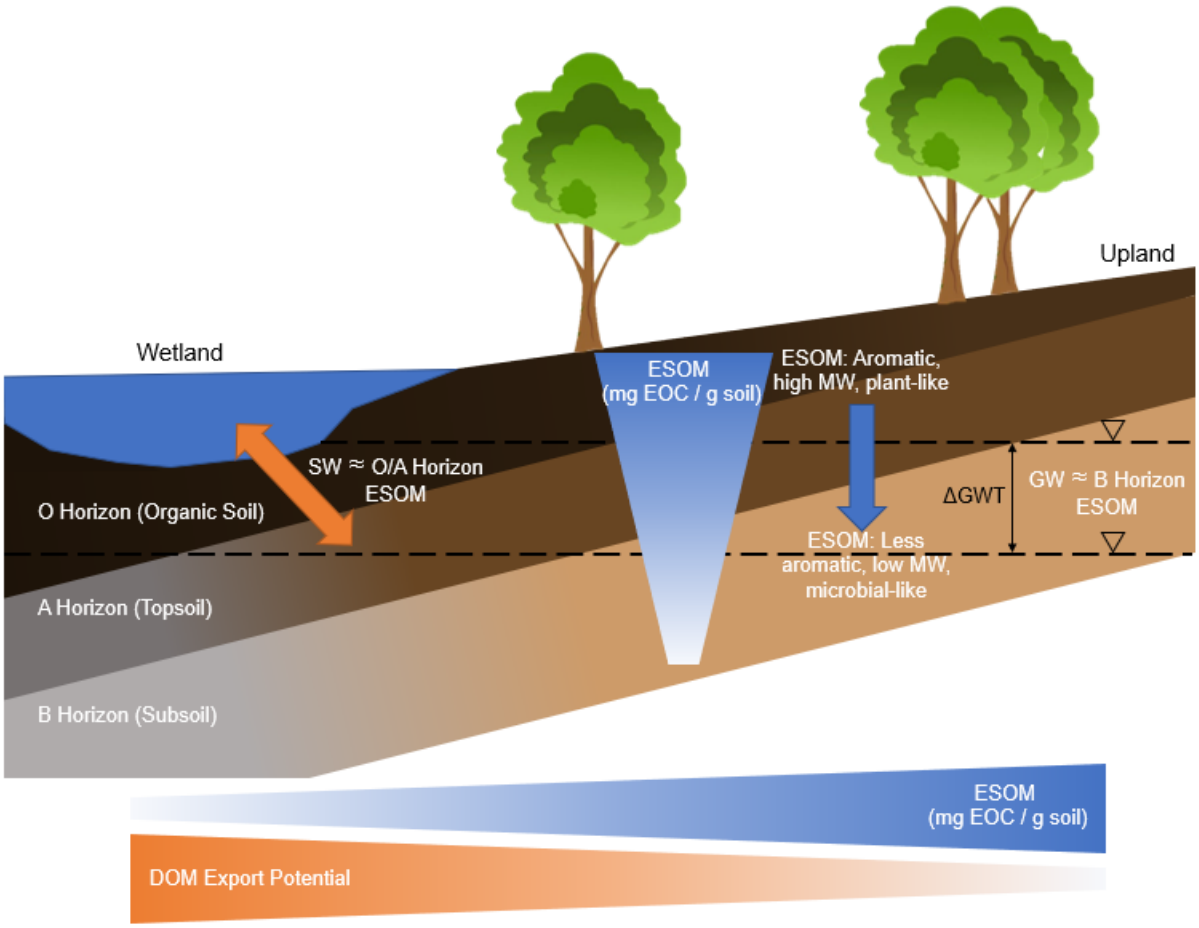

Figure 6.1: ESOM quantity and quality dynamics along upland to wetland transects in the Delmarva Bay GIW system.

Bibliography

Balcarczyk, K. L., Jones, J. B., Jaffé, R., and Maie, N. (2009). Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry*, 94(3):255–270.

Barnes, R. T., Butman, D. E., Wilson, H. F., and Raymond, P. A. (2018). Riverine Export of Aged Carbon Driven by Flow Path Depth and Residence Time. *Environmental Science and Technology*, 52(3):1028–1035.

Birkel, C., Duvert, C., Correa, A., Munksgaard, N. C., Maher, D. T., and Hutley, L. B. (2020). Tracer-Aided Modeling in the Low-Relief, Wet-Dry Tropics Suggests Water Ages and DOC Export Are Driven by Seasonal Wetlands and Deep Groundwater. *Water Resources Research*, 56(4):1–20.

Borken, W., Xu, Y. J., Brumme, R., and Lamersdorf, N. (1999). A Climate Change Scenario for Carbon Dioxide and Dissolved Organic Carbon Fluxes from a Temperate Forest Soil Drought and Rewetting Effects. *Soil Science Society of America Journal*, 63(6):1848–1855.

Bouyoucos, G. J. (1936). Directions for making mechanical analyses of soils by the hydrometer method. *Soil Science*, 42(3):225–230.

Boyer, E. W., Hornberger, M., G., Bencala, K. E., and McKnight, D. M. (1995). Variation of dissolved organic carbon during snowmelt in soil and stream waters of two headwater catchments, Summit County, Colorado. *Biogeochemistry of seasonally snow-covered catchments. Proc. symposium, Boulder, 1995*, 228(228):303–312.

Capps, K. A., Rancatti, R., Tomczyk, N., Parr, T. B., Calhoun, A. J., and Hunter, M.

(2014). Biogeochemical Hotspots in Forested Landscapes: The Role of Vernal Pools in Denitrification and Organic Matter Processing. *Ecosystems*, 17(8):1455–1468.

Catalán, N., Marcé, R., Kothawala, D. N., and Tranvik, L. J. (2016). Organic carbon decomposition rates controlled by water retention time across inland waters. *Nature Geoscience*, 9(7):501–504.

Chantigny, M. H. (2003). Dissolved and water-extractable organic matter in soils: A review on the influence of land use and management practices. *Geoderma*, 113(3-4):357–380.

Chen, C., Hall, S. J., Coward, E., and Thompson, A. (2020). Iron-mediated organic matter decomposition in humid soils can counteract protection. *Nature Communications*, 11(1):1–13.

Chow, A. T., Tanji, K. K., Gao, S., and Dahlgren, R. A. (2006). Temperature, water content and wet-dry cycle effects on DOC production and carbon mineralization in agricultural peat soils. *Soil Biology and Biochemistry*, 38(3):477–488.

Christ, M. J. and David, M. B. (1996). Temperature and moisture effects on the production of dissolved organic carbon in a Spodosol. *Soil Biology and Biochemistry*, 28(9):1191–1199.

Cincotta, M., Perdrial, J., Shavitz, A., Libenson, A., Landsman-Gerjoi, M., Perdrial, N., Armfield, J., Adler, T., and Shanley, J. (2019). Soil Aggregates as a Source of Dissolved Organic Carbon to Streams: An Experimental Study on the Effect of Solution Chemistry on Water Extractable Carbon. *Frontiers in Environmental Science*, 7(November):1–15.

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, 51(4):325–346.

Cohen, M. J., Creed, I. F., Alexander, L., Basu, N. B., Calhoun, A. J., Craft, C., D'Amico, E., DeKeyser, E., Fowler, L., Golden, H. E., Jawitz, J. W., Kalla, P., Kirkman, L. K.,

Lane, C. R., Lang, M., Leibowitz, S. G., Lewis, D. B., Marton, J., McLaughlin, D. L., Mushet, D. M., Raanan-Kiperwas, H., Rains, M. C., Smith, L., and Walls, S. C. (2016). Do geographically isolated wetlands influence landscape functions? *Proceedings of the National Academy of Sciences of the United States of America*, 113(8):1978–1986.

Corvasce, M., Zsolnay, A., D'Orazio, V., Lopez, R., and Miano, T. M. (2006). Characterization of water extractable organic matter in a deep soil profile. *Chemosphere*, 62(10):1583–1590.

Cory, R. M. and McKnight, D. M. (2005). Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental Science and Technology*, 39(21):8142–8149.

Covino, T. (2017). Hydrologic connectivity as a framework for understanding biogeochemical flux through watersheds and along fluvial networks. *Geomorphology*, 277:133–144.

Creed, I. F., Sanford, S. E., Beall, F. D., Molot, L. A., and Dillon, P. J. (2003). Cryptic wetlands: Integrating hidden wetlands in regression models of the export of dissolved organic carbon from forested landscapes. *Hydrological Processes*, 17(18):3629–3648.

D'Amore, D. V., Fellman, J. B., Edwards, R. T., and Hood, E. (2010). Controls on dissolved organic matter concentrations in soils and streams from a forested wetland and sloping bog in southeast Alaska. *Ecohydrology*, 3(February):249–261.

Day, P. R. (1950). Physical basis of particle size analysis by the hydrometer method. *Soil Science*, 70(5):363–374.

Day, P. R. (1953). Experimental confirmation of hydrometer theory. *Soil Science*, 75(3):181–186.

Duan, X., Yu, X., Li, Z., Wang, Q., Liu, Z., and Zou, Y. (2020). Iron-bound organic carbon is conserved in the rhizosphere soil of freshwater wetlands. *Soil Biology and Biochemistry*, 149:107949.

Duston, S. A. (2020). Capturing and Characterizing Soluble Organic Matter Dynamics in Soil Formation Processes.

Epting, S. M., Alec, W., Hosen, J. D., Palmer, M. A., Alexander, L. C., and Lang, M. W. (2018). Landscape metrics as predictors of hydrologic connectivity between Coastal Plain forested wetlands and streams. (December 2017):516–532.

Evans, C. D., Jones, T. G., Burden, A., Ostle, N., Zieliński, P., Cooper, M. D., Peacock, M., Clark, J. M., Oulehle, F., Cooper, D., and Freeman, C. (2012). Acidity controls on dissolved organic carbon mobility in organic soils. *Global Change Biology*, 18(11):3317–3331.

Fellman, J. B., D'Amore, D. V., Hood, E., and Boone, R. D. (2008). Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. *Biogeochemistry*, 88(2):169–184.

Fellman, J. B., Hood, E., Edwards, R. T., and D'Amore, D. V. (2009). Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. *Journal of Geophysical Research: Biogeosciences*, 114(1):1–14.

Fellman, J. B., Hood, E., and Spencer, R. G. (2010). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology and Oceanography*, 55(6):2452–2462.

Fenstermacher, D. (2012). *Carbon Storage and Potential Carbon Sequestration in Depressional Wetlands of the Mid-Atlantic Region*. PhD thesis, University of Maryland.

Fenstermacher, D. E., Rabenhorst, M. C., Lang, M. W., McCarty, G. W., and Needelman, B. A. (2014). Distribution, morphometry, and land use of Delmarva Bays. *Wetlands*, 34(6):1219–1228.

Fenstermacher, D. E., Rabenhorst, M. C., Lang, M. W., McCarty, G. W., and Needelman, B. A. (2016). Carbon in Natural, Cultivated, and Restored Depressional Wetlands in the Mid-Atlantic Coastal Plain. *Journal of Environmental Quality*, 45(2):743–750.

Fiedler, S. and Kalbitz, K. (2003). Concentrations and properties of dissolved organic matter in forest soils as affected by the redox regime. *Soil Science*, 168(11):793–801.

Gabor, R. S., Burns, M. A., Lee, R. H., Elg, J. B., Kemper, C. J., Barnard, H. R., and McKnight, D. M. (2015). Influence of leaching solution and catchment location on the fluorescence of water-soluble organic matter. *Environmental Science and Technology*, 49(7):4425–4432.

Grybos, M., Davranche, M., Gruau, G., Petitjean, P., and Pédrot, M. (2009). Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. *Geoderma*, 154(1-2):13–19.

Helms, R. J., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., and Mopper, K. (2009). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter (Limnology and Oceanography 53 955-969). *Limnology and Oceanography*, 54(3):1023.

Hondula, K. L., Jones, C. N., and Palmer, M. (2021). Effects of seasonal inundation on methane fluxes from forested freshwater wetlands. *Environmental Research Letters*.

Hosen, J. D., Armstrong, A. W., and Palmer, M. A. (2018). Dissolved organic matter variations in coastal plain wetland watersheds: The integrated role of hydrological connectivity, land use, and seasonality. *Hydrological Processes*, 32(11):1664–1681.

Hoyt, A. M., Gandois, L., Eri, J., Kai, F. M., Harvey, C. F., and Cobb, A. R. (2019). Co2 emissions from an undrained tropical peatland: Interacting influences of temperature, shading and water table depth. *Global change biology*, 25(9):2885–2899.

Inamdar, S., Finger, N., Singh, S., Mitchell, M., Levia, D., Bais, H., Scott, D., and McHale, P. (2012). Dissolved organic matter (dom) concentration and quality in a forested mid-atlantic watershed, usa. *Biogeochemistry*, 108(1):55–76.

Jeanneau, L., Buysse, P., Denis, M., Gruau, G., Petitjean, P., Jaffrézic, A., Flechard, C., and Viaud, V. (2020). Water table dynamics control carbon losses from the destabilization of soil organic matter in a small, lowland agricultural catchment. *Soil Systems*, 4(1):1–17.

Jones, D. L. and Willett, V. B. (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, 38(5):991–999.

Jones, J. B. and Stanley, E. (2016). *Stream ecosystems in a changing environment*. Elsevier.

Kaiser, K. and Guggenberger, G. (2000). The role of dom sorption to mineral surfaces in the preservation of organic matter in soils. *Organic geochemistry*, 31(7-8):711–725.

Kaiser, K. and Kalbitz, K. (2012). Cycling downwards - dissolved organic matter in soils. *Soil Biology and Biochemistry*, 52:29–32.

Kaiser, M., Kleber, M., and Berhe, A. A. (2015). How air-drying and rewetting modify soil organic matter characteristics: An assessment to improve data interpretation and inference. *Soil Biology and Biochemistry*, 80:324–340.

Kalbitz, K., Solinger, S., Park, J. H., Michalzik, B., and Matzner, E. (2000). Controls on the dynamics dissolved organic matter in soils: A review. *Soil Science*, 165(4):277–304.

Kassambara, A. and Mundt, F. (2020). *factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. R package version 1.0.7.

Kayranli, B., Scholz, M., Mustafa, A., and Hedmark, Å. (2010). Carbon storage and fluxes within freshwater wetlands: A critical review. *Wetlands*, 30(1):111–124.

Knorr, K. H. (2013). DOC-dynamics in a small headwater catchment as driven by redox fluctuations and hydrological flow paths - Are DOC exports mediated by iron reduction/oxidation cycles? *Biogeosciences*, 10(2):891–904.

Kottkamp, A. I. (2019). Hydrologic Drivers of Soil Organic Carbon Storage and Stability in Freshwater Mineral Wetlands.

Lacroix, R. E., Tfaily, M. M., McCreight, M., Jones, M. E., Spokas, L., and Keiluweit, M. (2019). Shifting mineral and redox controls on carbon cycling in seasonally flooded mineral soils. *Biogeosciences*, 16(13):2573–2589.

Lane, C. R., Leibowitz, S. G., Autrey, B. C., LeDuc, S. D., and Alexander, L. C. (2018). Hydrological, Physical, and Chemical Functions and Connectivity of Non-Floodplain Wetlands to Downstream Waters: A Review. *Journal of the American Water Resources Association*, 54(2):346–371.

Lee, S., McCarty, G. W., Moglen, G. E., Lang, M. W., Nathan Jones, C., Palmer, M., Yeo, I. Y., Anderson, M., Sadeghi, A. M., and Rabenhorst, M. C. (2020). Seasonal drivers of geographically isolated wetland hydrology in a low-gradient, Coastal Plain landscape. *Journal of Hydrology*, 583(January).

Lee, S., McCarty, G. W., Moglen, G. E., Lang, M. W., Sadeghi, A. M., Green, T. R., Yeo, I.-Y., and Rabenhorst, M. C. (2019). Effects of subsurface soil characteristics on wetland–groundwater interaction in the coastal plain of the chesapeake bay watershed. *Hydrological Processes*, 33(2):305–315.

Lehmann, J. and Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, 528(7580):60–68.

Ma, W., Li, Z., Ding, K., and Zhou, Q. (2021). Dynamics of water extractable organic carbon at a subtropical catchment using fluorescence excitation-emission matrix spectroscopy coupled with parallel factor analysis. *European Journal of Soil Science*, 72(2):871–885.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., and Hornik, K. (2021). *cluster: Cluster Analysis Basics and Extensions*. R package version 2.1.2 — For new features, see the 'Changelog' file (in the package source).

Maietta, C. E., Hondula, K. L., Jones, C. N., and Palmer, M. A. (2020). Hydrological Conditions Influence Soil and Methane-Cycling Microbial Populations in Seasonally Saturated Wetlands. *Frontiers in Environmental Science*, 8(November):1–13.

Marton, J. M., Creed, I. F., Lewis, D. B., Lane, C. R., Basu, N. B., Cohen, M. J., and Craft, C. B. (2015). Geographically isolated wetlands are important biogeochemical reactors on the landscape. *BioScience*, 65(4):408–418.

McDonough, O. T., Lang, M. W., Hosen, J. D., and Palmer, M. A. (2015). Surface Hydrologic Connectivity Between Delmarva Bay Wetlands and Nearby Streams Along a Gradient of Agricultural Alteration. *Wetlands*, 35(1):41–53.

McDowell, W. H., Zsolnay, A., Aitkenhead-Peterson, J. A., Gregorich, E. G., Jones, D. L., Jödemann, D., Kalbitz, K., Marschner, B., and Schwesig, D. (2006). A comparison of

methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. *Soil Biology and Biochemistry*, 38(7):1933–1942.

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T., and Andersen, D. T. (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, 46(1):38–48.

McLaughlin, D. L., Kaplan, D. A., and Cohen, M. J. (2014). A significant nexus: Geographically isolated wetlands influence landscape hydrology. *Water Resources Research*, 50(9):7153–7166.

Mitsch, W. J., Bernal, B., Nahlik, A. M., Mander, Ü., Zhang, L., Anderson, C. J., Jørgensen, S. E., and Brix, H. (2013). Wetlands, carbon, and climate change. *Landscape Ecology*, 28(4):583–597.

Müller, M., Alewell, C., and Hagedorn, F. (2009). Effective retention of litter-derived dissolved organic carbon in organic layers. *Soil Biology and Biochemistry*, 41(6):1066–1074.

Murphy, K. R., Stedmon, C. A., Graeber, D., and Bro, R. (2013a). Appendix A: drEEM. *Analytical Methods*, 5:Supplementary Information.

Murphy, K. R., Stedmon, C. A., Graeber, D., and Bro, R. (2013b). Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Analytical Methods*, 5(23):6557–6566.

Murphy, K. R., Stedmon, C. A., Wenig, P., and Bro, R. (2014). OpenFluor- An online spectral library of auto-fluorescence by organic compounds in the environment. *Analytical Methods*, 6(3):658–661.

Nature Geoscience (2021). Valuing wetlands. *Nature Geoscience*, 14(3):111–111.

Neff, B. P., Rosenberry, D. O., Leibowitz, S. G., Mushet, D. M., Golden, H. E., Rains, M. C., Brooks, J. R., and Lane, C. R. (2020). A Hydrologic Landscapes Perspective on Groundwater Connectivity of Depressional Wetlands. *Water*.

NRCS (2006). Field Indicators of Hydric Soils in the United States. 0:47.

Ohno, T. (2002). Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science and Technology*, 36(4):742–746.

Pallud, C., Rhoades, C. C., Schneider, L., Dwivedi, P., and Borch, T. (2020). *Temperature-induced iron (III) reduction results in decreased dissolved organic carbon export in subalpine wetland soils, Colorado, USA*, volume 280. Elsevier Ltd.

Phillips, P. J. and Shedlock, R. J. (1993). Hydrology and chemistry of groundwater and seasonal ponds in the Atlantic Coastal Plain in. *Journal of Hydrology*, 41:157–178.

Pringle, C. (2003). What is hydrologic connectivity and why is it ecologically important? *Hydrological Processes*, 17(13):2685–2689.

PRISM Climate Group (2004). Oregon State University. *PRISM Climate Data*.

R Core Team (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rains, M. C., Leibowitz, S. G., Cohen, M. J., Creed, I. F., Golden, H. E., Jawitz, J. W., Kalla, P., Lane, C. R., Lang, M. W., and Mclaughlin, D. L. (2016). Geographically isolated wetlands are part of the hydrological landscape. *Hydrological Processes*, 30(1):153–160.

Reddy, K. R. and Patrick, W. H. (1975). Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. *Soil Biology and Biochemistry*, 7(2):87–94.

Rennert, T., Gockel, K. F., and Mansfeldt, T. (2007). Extraction of water-soluble organic matter from mineral horizons of forest soils. *Journal of Plant Nutrition and Soil Science*, 170(4):514–521.

Roth, V. N., Lange, M., Simon, C., Hertkorn, N., Bucher, S., Goodall, T., Griffiths, R. I., Mellado-Vázquez, P. G., Mommer, L., Oram, N. J., Weigelt, A., Dittmar, T., and Gleixner, G. (2019). Persistence of dissolved organic matter explained by molecular changes during its passage through soil. *Nature Geoscience*, 12(9):755–761.

Rouwane, A., Grybos, M., Bourven, I., Rabiet, M., and Guibaud, G. (2018). Waterlogging and soil reduction affect the amount and apparent molecular weight distribution of dissolved organic matter in wetland soil: A laboratory study. *Soil Research*, 56(1):28–38.

Saraceno, J. F., Pellerin, B. A., Downing, B. D., Boss, E., Bachand, P. A., and Bergamaschi, B. A. (2009). High-frequency in situ optical measurements during a storm event: Assessing relationships between dissolved organic matter, sediment concentrations, and hydrologic processes. *Journal of Geophysical Research: Biogeosciences*, 114(4):1–11.

Schiff, S., Aravena, R., Mewhinney, E., Elgood, R., Warner, B., Dillon, P., and Trumbore, S. (1998). Precambrian shield wetlands: hydrologic control of the sources and export of dissolved organic matter. *Climatic Change*, 40(2):167–188.

Shen, Y., Chapelle, F. H., Strom, E. W., and Benner, R. (2015). Origins and bioavailability of dissolved organic matter in groundwater. *Biogeochemistry*, 122(1):61–78.

Singh, S., Inamdar, S., Mitchell, M., and McHale, P. (2014). Seasonal pattern of dissolved organic matter (DOM) in watershed sources: Influence of hydrologic flow paths and autumn leaf fall. *Biogeochemistry*, 118(1-3):321–337.

Soil Survey Staff, U. (2017). Web soil survey.

Thompson, Y., Sandefur, B. C., Karathanasis, A. D., and D'Angelo, E. (2009). Redox potential and seasonal porewater biogeochemistry of three mountain wetlands in Southeastern Kentucky, USA. *Aquatic Geochemistry*, 15(3):349–370.

Tiner, R. W. (2003). Geographically isolated wetlands of the United States. *Wetlands*, 23(3):494–516.

Villa, J. A. and Bernal, B. (2018). Carbon sequestration in wetlands, from science to practice: An overview of the biogeochemical process, measurement methods, and policy framework. *Ecological Engineering*, 114:115–128.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K. (2003). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology*, 37(20):4702–4708.

Werner, B. J., Musolff, A., Lechtenfeld, O. J., Rooij, G. H. D., Oosterwoud, M. R., and Fleckenstein, J. H. (2019). High-frequency measurements explain quantity and quality of dissolved organic carbon mobilization in a headwater catchment. *Biogeosciences*, 16(22):4497–4516.

Wolf, K. L., Ahn, C., and Noe, G. B. (2011). Development of soil properties and nitrogen cycling in created wetlands. *Wetlands*, 31(4):699–712.

Zhao, Q., Dunham-Cheatham, S., Adhikari, D., Chen, C., Patel, A., Poulson, S. R., Obrist, D., Verburg, P. S., Wang, X., Roden, E. R., Thompson, A., and Yang, Y. (2020). Oxidation of soil organic carbon during an anoxic-oxic transition. *Geoderma*, 377(July):114584.

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., and Saccomandi, F. (1999). Differ-

entiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere*, 38(1):45–50.

Appendices

Appendix A

Supplemental Information

This section includes additional information related to site characteristics, ESOM results, and PAPRAFAC modeling referred to in the Thesis Results and Discussion above.

A.1 Transect Morphology and Hydrology

Figures A.1, A.2, and A.3 show soil horizon depths and distance along the transect for Wetland sites 2, 3, and 4. Figure A.4 displays mean water level by transect point and with mean soil depths overlaid to visualize water table movement through the soil horizons during the 2020 water year.

A.2 ESOM

Table A.1 shows results from paired t-tests used to verify that ESOM quantity and quality values were consistent between the two sampling campaigns. Depth of sample captured at the Wetland and Edge varied between the two sampling campaigns due to difficulty gathering samples in inundated conditions. Therefore, Wetland O and A horizons were grouped together during the paired t-test to ensure there was a sufficient number of samples, while Wetland and Edge B horizons were eliminated due to an insufficient number of samples. Figure A.5 shows k-means cluster analyses used to identify groupings in data

comparing $SUVA_{254}$ and FI to ESOM quantity (EOC). Table A.2 presents fluorescence metrics from other ESOM and DOM studies, demonstrating that results from this study are reasonable for this upland to wetland soil environment.

A.3 PARAFAC Model Information

Table A.3 provides descriptions and probable sources of the notable components from the Cory and McKnight PARAFAC model (Cory and McKnight, 2005). Table A.4 presents results from paired t-tests used to evaluate consistency of sampling loadings between sampling campaigns. Similar to the ESOM fluorescence metrics, Wetland O and A horizons were grouped together to ensure a sufficient number of samples for paired t-tests and Wetland and Edge B horizons were eliminated due to an insufficient number of samples. Table A.5 presents descriptions and probable sources of components from the Delmarva PARAFAC model identified using the OpenFluor database (Murphy et al., 2014). Figure A.6 shows sampling loadings using the Delmarva PARAFAC model. Figure A.7 demonstrates the lack of trends along the transect and within soil horizons.

A.4 Between Site Variation

Figure A.8 provides individual hydrographs for each wetland site during the 2020 water year. All four wetlands have similar trends throughout the water year where the sites were dry in October 2019, wetted up through spring 2020, and then experienced fluctuating water levels in late summer and early fall. Wetlands 1 and 2 have higher water levels at the Wetland and Edge points and lower water levels at the Transition and Upland points compared to Wetlands 3 and 4. Figures A.9, A.10, and A.11 provide additional plots

demonstrating the increased variability in ESOM results at Wetlands 1 and 2 as a result of morphological differences creating drier conditions relative to Wetlands 3 and 4.

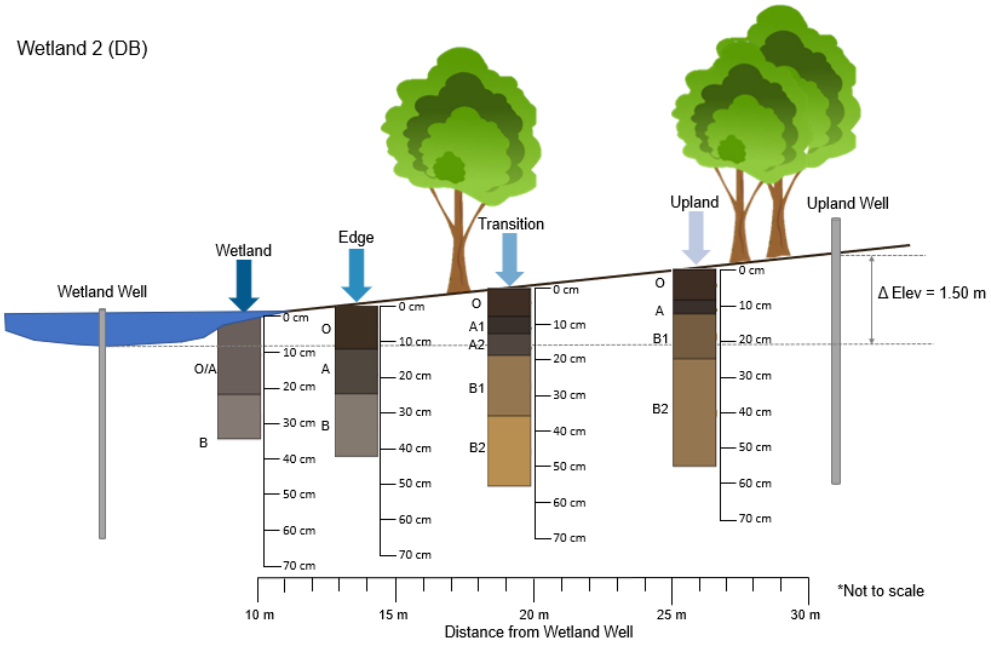

Figure A.1: Soil horizon depths (y-axis) and distance from wetland center along the transect (x-axis) at Wetland 2 (DB).

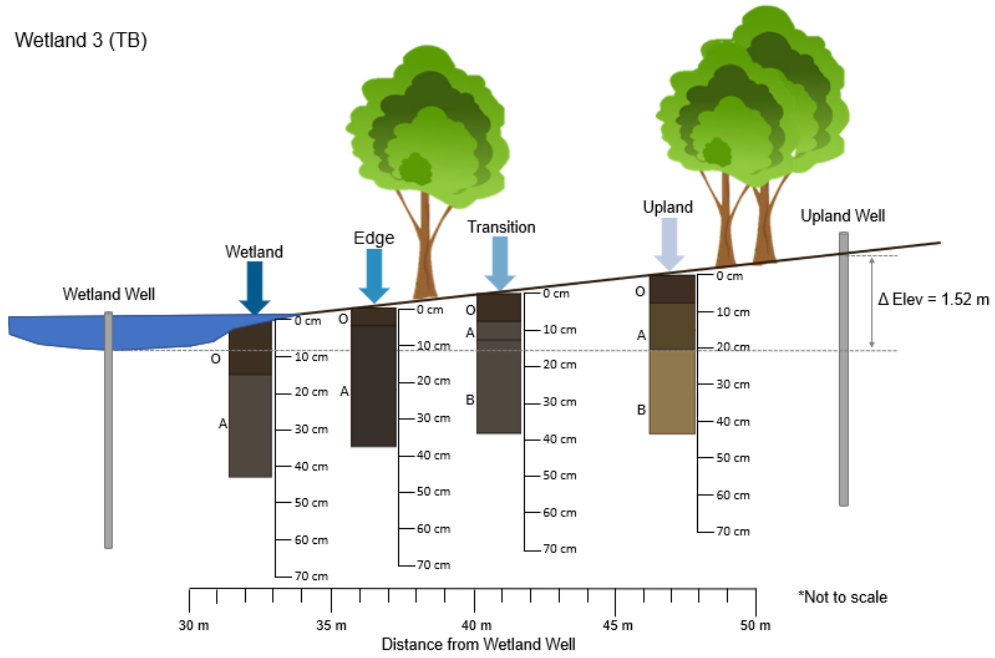

Figure A.2: Soil horizon depths (y-axis) and distance from wetland center along the transect (x-axis) at Wetland 3 (TB).

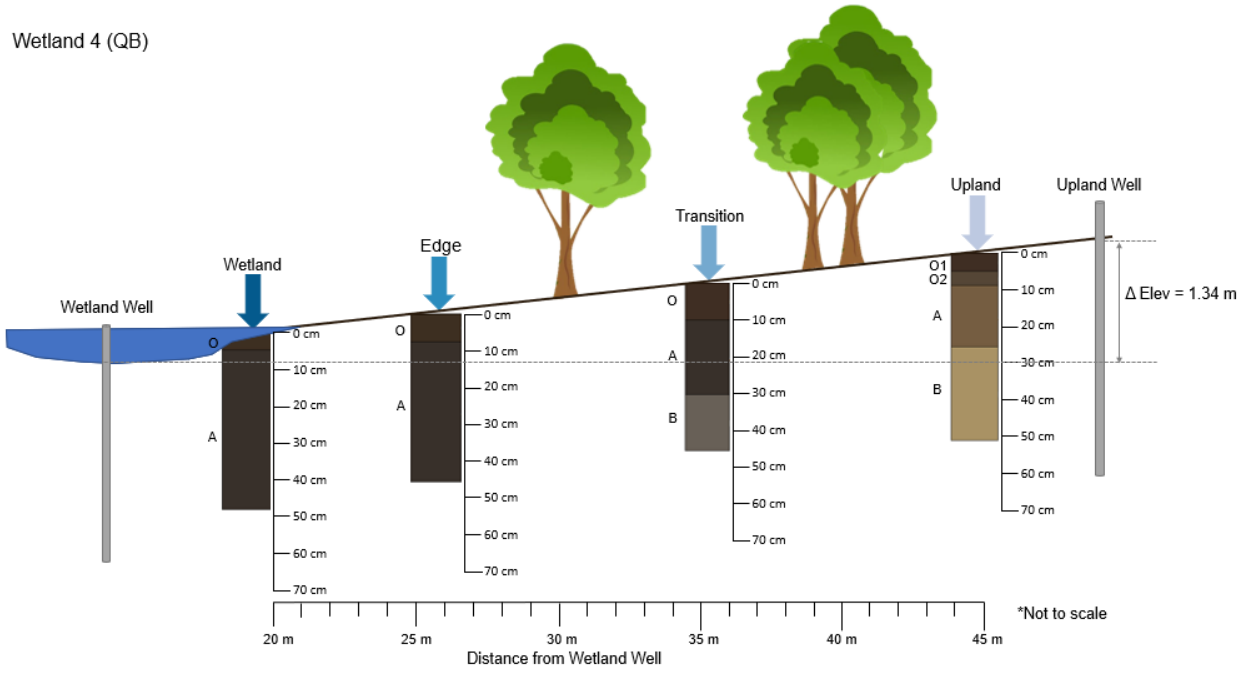

Figure A.3: Soil horizon depths (y-axis) and distance from wetland center along the transect (x-axis) at Wetland 4 (QB).

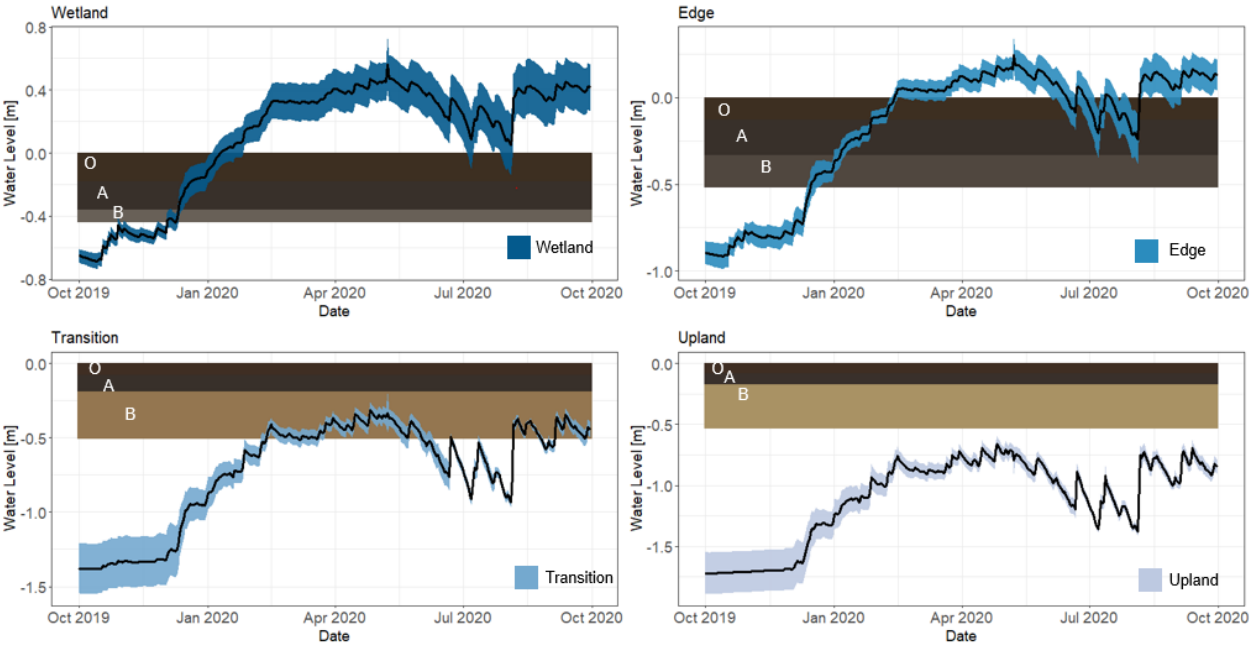

Figure A.4: Hydrograph of mean water level at each transect point across the four sites. Colored ribbons represent standard error. Mean soil horizon depths are overlaid in shades of brown.

Table A.1: Paired t-test results for ESOM quantity and fluorescence metrics.

			ESOM (mg EOC / g soil)		FI		$SUVA_{254}$ (L/mg·m)		HIX		SSR	
Location	**Horizon**	**df**	**t-statistic**	**p-value**	**t-statistic**	**p-value**	**t-statistic**	**p-value**	**t-statistic**	**p-value**	**t-statistic**	**p-value**
Wetland	O/A	3	-2.61	0.08	-0.13	0.90	0.69	0.54	4.68	0.018	-0.80	0.48
Edge	O	3	-1.56	0.22	0.15	0.89	1.33	0.28	2.89	0.063	-3.31	0.05
	A	2	-0.83	0.49	0.64	0.59	-0.93	0.45	-0.73	0.54	1.48	0.28
Transition	O	3	0.98	0.40	-1.44	0.25	0.11	0.92	0.089	0.94	-1.37	0.26
	A	5	0.26	0.81	0.87	0.43	-0.91	0.40	1.15	0.30	0.55	0.61
	B	4	-0.36	0.74	-0.50	0.64	-0.45	0.68	0.77	0.48	-2.12	0.12
Upland	O	3	-0.26	0.81	1.09	0.35	2.68	0.08	2.28	0.11	-10.1	0.002
	A	3	-1.08	0.36	1.26	0.30	-1.62	0.20	-0.89	0.44	-0.28	0.80
	B	4	1.17	0.31	-0.57	0.60	-0.25	0.82	-0.25	0.82	-1.49	0.23

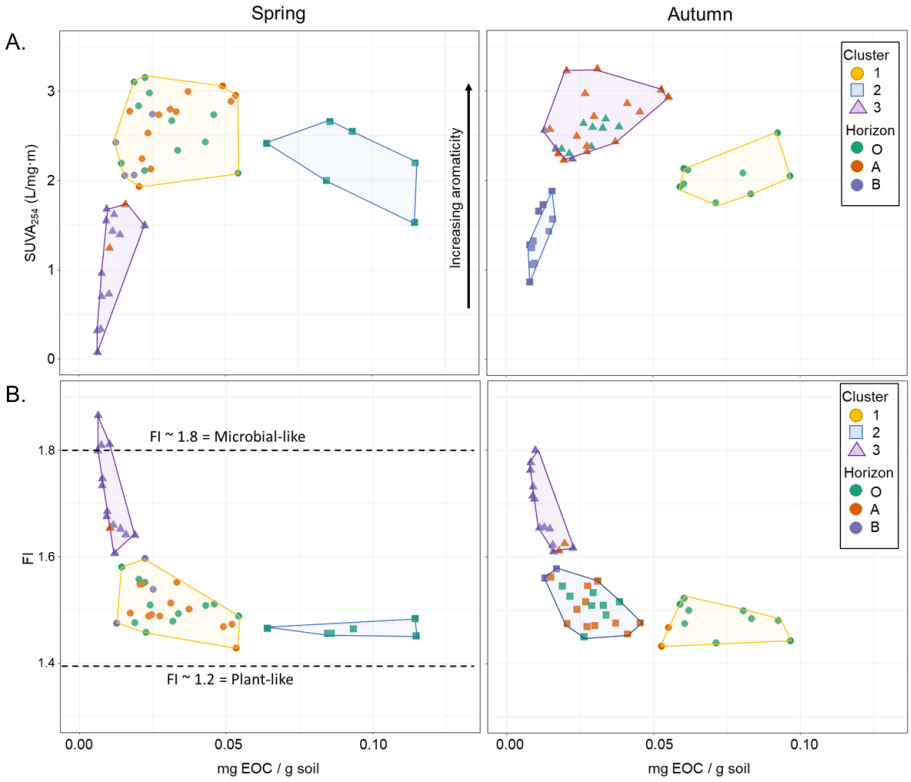

Figure A.5: (A) $SUVA_{254}$ vs EOC and (B) FI vs EOC over the two sampling campaigns. Shape indicates k-means cluster grouping and color indicates soil horizon.

Table A.2: Fluorescence metrics from previous ESOM and DOM studies compared to results from this study.

		Fluorescence Metric	SUVA254 (L/mg·m)		FI		HIX		SSR	
Study	Study Type	Study System	Approximate Range	Notes	Approximate Range	Notes	Approximate Range	Notes	Approximate Range	Notes
Wardinski, (2021)	ESOM	Delmarva Bays, Maryland, USA	0.5 - 3	Decrease with depth	1.4 – 1.8	Increase with depth	0.35 – 0.60	Highest in shallow soils	1.2 - 4	Increase with depth
Gabor et al., (2015)	ESOM	Forest/ Riparian soils, Colorado, USA	0 - 25	-	1.2 – 1.6	-	-	-	-	-
Corvasce et al., (2006)	ESOM	High plain soils, Italy	-	-	-	-	0.5 – 4	Highest in A horizon	-	-
Ma et al., (2020)	ESOM	Subtropical, mixed land use, China	-	-	1.6 – 2.2	Increase with depth	0.1 – 0.6	Highest in shallow soils	1 – 7	Increase with depth
Cincotta et al., (2019)	ESOM	Headwater catchment, Vermont, USA	-	-	1.2 – 2.0	-	2 – 7	-	-	-
Weishaar et al., (2003)	DOM	Whole water samples	0.6 – 5.3	SUVA254 foundational paper	-	-	-	-	-	-
McKnight et al., (2001)	DOM	Surface water samples	-	-	1.4 – 2.0	FI foundational paper	-	-	-	-
Ohno, (2002)	DOM	Water soluble DOM	-	-	-	-	3 - 8	HIX foundational paper	-	-
Helms et al., (2008)	DOM	Whole water samples	-	-	-	-	-	-	0.5 – 2.5	SSR foundational paper
Fellman et al., (2008)	DOM	Forest/wetland soils, Alaska, USA	3.4 – 4.4	-	-	-	-	-	-	-
Inamdar et al., (2012)	DOM	Wetland soil water, Mid-Atlantic watershed, USA	3 - 6	-	~1.4		0.8 – 0.9	-	1.5 – 6	-
Grybos et al., (2009)	DOM	Wetland soil	3 – 5.5	-	-	-	-	-	-	-
Rouwane et al., (2018)	DOM	Wetland soil	4 – 10	-	-	-	-	-	-	-

Table A.3: Descriptions of components from the Cory and McKnight PARAFAC model that had highest loadings across the two sampling campaigns.

Notable Components	Ex/Em maxima (nm)	Probable Sources	Description
C1	340/450	T	High MW, humic, widespread, high in wetlands and forested environments
C2 (Q2)	250/510	T	Oxidized, quinone-like, high MW, aromatic humic, highest in wetlands and forested environments
C4 (HQ)	250/550	T, M	Reduced, quinone-like, humic like, correlated with %anomeric/acetal/ketal carbon
C8 (Tryptophan)	270/360	T, A, M	Amino acids, free or bound in proteins, indicates less degraded peptide material
C11 (Q1)	260/538	T	Fluvic acid, wide-spread
C12 (Q3)	250/388	A, M	Oxidized, quinone-like, humic-like, correlated with aliphatic C content, autochthonous production, potential photoproduct of terrestrial DOM
C13 (Tyrosine)	280/350	T, A, M	Amino acids, free or bound in proteins, indicates more degraded peptide material

Table A.4: Paired t-test results for the notable components of the Cory and McKnight PARAFAC model.

			C2		C4		C8		C12		C13	
Location	**Horizon**	**df**	**t-statistic**	**p-value**	**t-statistic**	**p-value**	**t-statistic**	**p-value**	**t-statistic**	**p-value**	**t-statistic**	**p-value**
Wetland	O/A	3	1.53	0.22	-0.29	0.79	-0.99	0.40	-0.53	0.63	0.40	0.71
Edge	O	3	2.16	0.12	-1.92	0.15	1.00	0.39	0.87	0.45	-4.60	0.019
	A	2	-0.71	0.55	-4.08	0.06	1.12	0.38	1.00	0.42	-1.60	0.25
Transition	O	3	0.65	0.56	-1.61	0.21	0.34	0.76	-0.09	0.93	-0.03	0.98
	A	5	1.82	0.13	-2.61	0.05	NA	NA	0.98	0.37	-1.37	0.23
	B	4	0.32	0.77	-0.82	0.46	-0.043	0.97	0.05	0.96	-0.82	0.46
Upland	O	3	2.75	0.07	-2.60	0.08	-1.79	0.17	1.42	0.25	-2.20	0.12
	A	3	-0.37	0.74	-7.67	0.004	1.00	0.39	1.50	0.23	-0.86	0.45
	B	4	-0.55	0.61	0.04	0.97	0.043	0.97	0.57	0.60	0.14	0.90

Table A.5: Descriptions of the four components identified in the Delmarva Synoptic PARAFAC model. Descriptions were gathered using the OpenFluor database where the studies cited had a 99 percent or better match to the Delmarva model excitation and emission wavelengths.

Component	Ex/Em maxima (nm)	Probable Sources	Description	References from OpenFluor Database
C1	261/437	T	Terrestrial origin, humic fluorescence, fluvic acid	Lambert et al (2016), Stedmon et al (2007), Stedmon and Markager (2005), Wauthy et al (2018)
C2	261/497	T	Aromatic carbon, terrestrial sources, susceptible to photochemical and microbial degradation, intermediate of terrestrial DOM degradation	Cory and McKnight (2005), Stedmon et al (2007), Wauthy et al (2018), Graeber et al (2012)
C3	261/384	M, A	Microbial-like, autochthonous primary production	Lambert et al (2016), Osburn et al (2015)
C4	261/329	M	Protein-like, tryptophan signature, amino acids	Groeneveld et al (2020), Crawley et al (2012), Yamashita et al (2010)

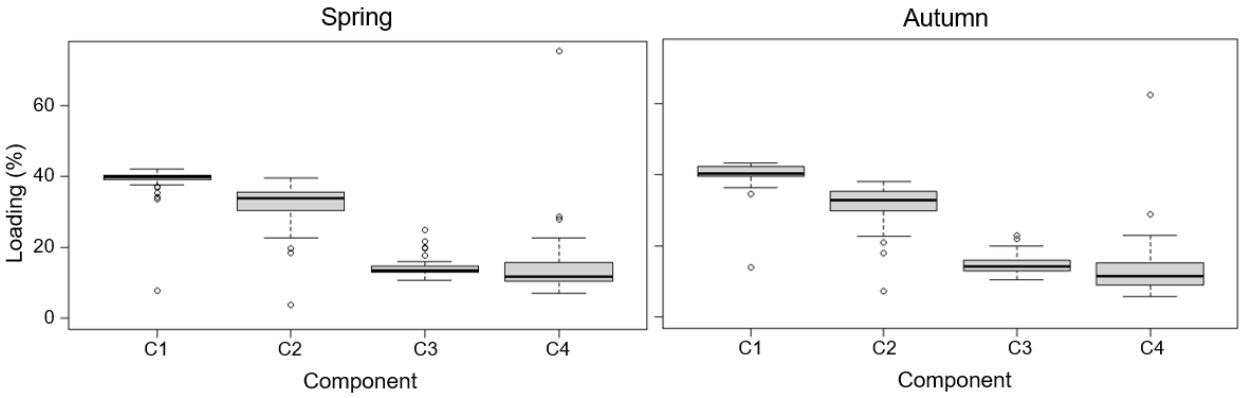

Figure A.6: ESOM sampling loadings for the four component Delmarva Synoptic PARAFAC model during the two sampling campaigns.

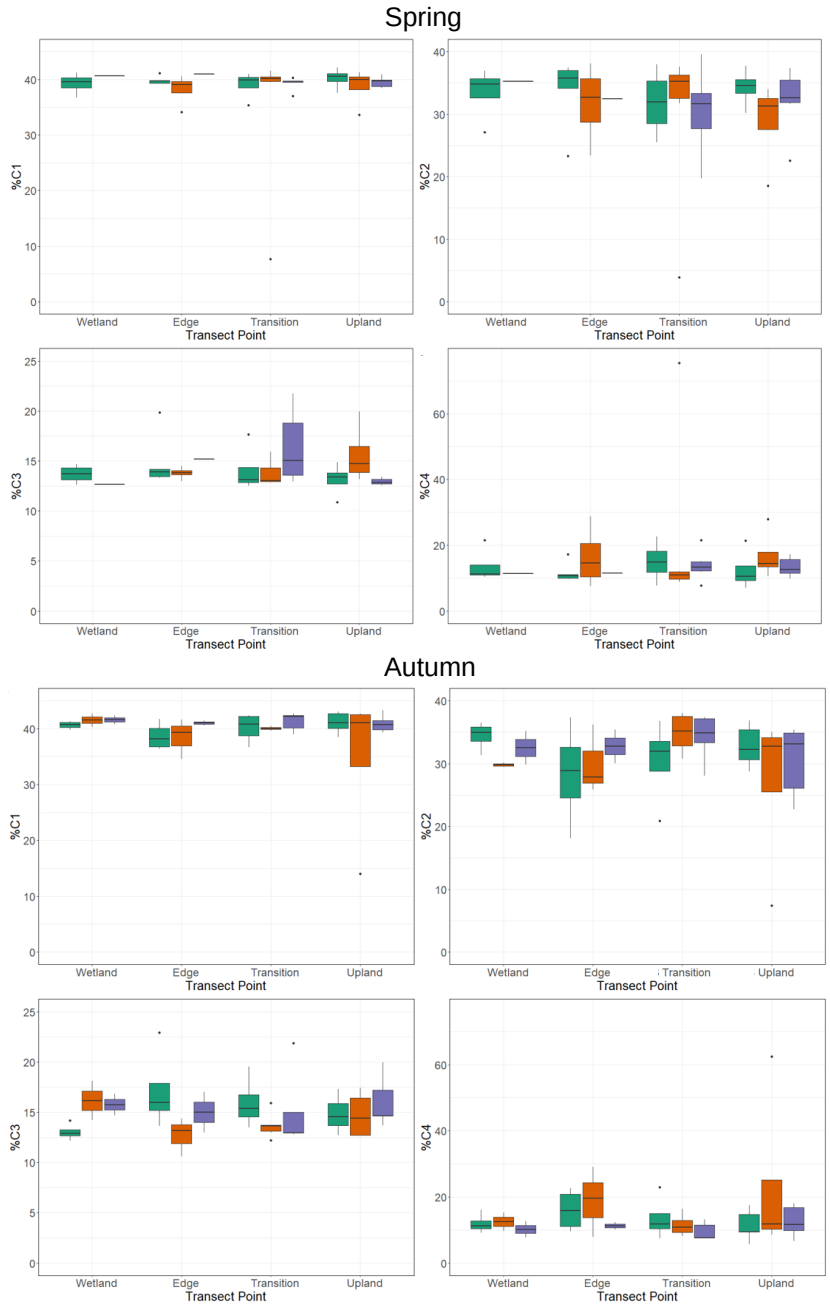

Figure A.7: ESOM sample loadings for each soil horizon and transect point across all four wetland sites using the Delmarva PARAFAC model.

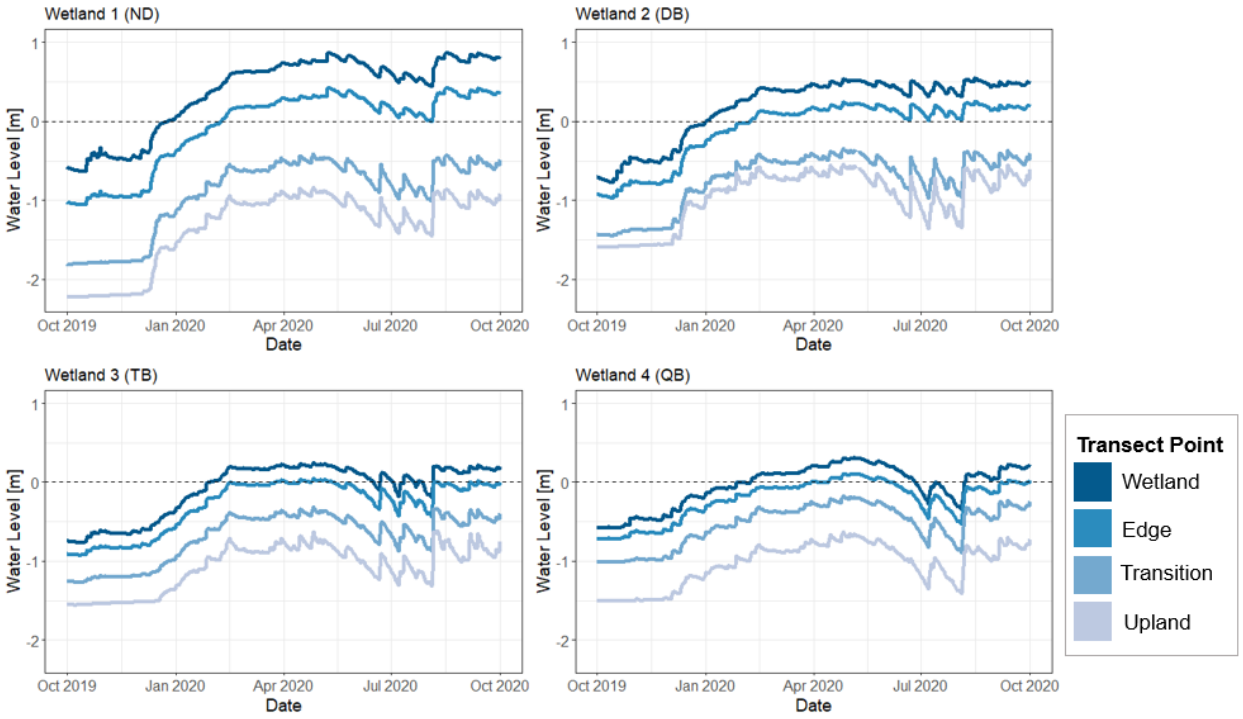

Figure A.8: Hydrographs for each of the four wetlands sampled.

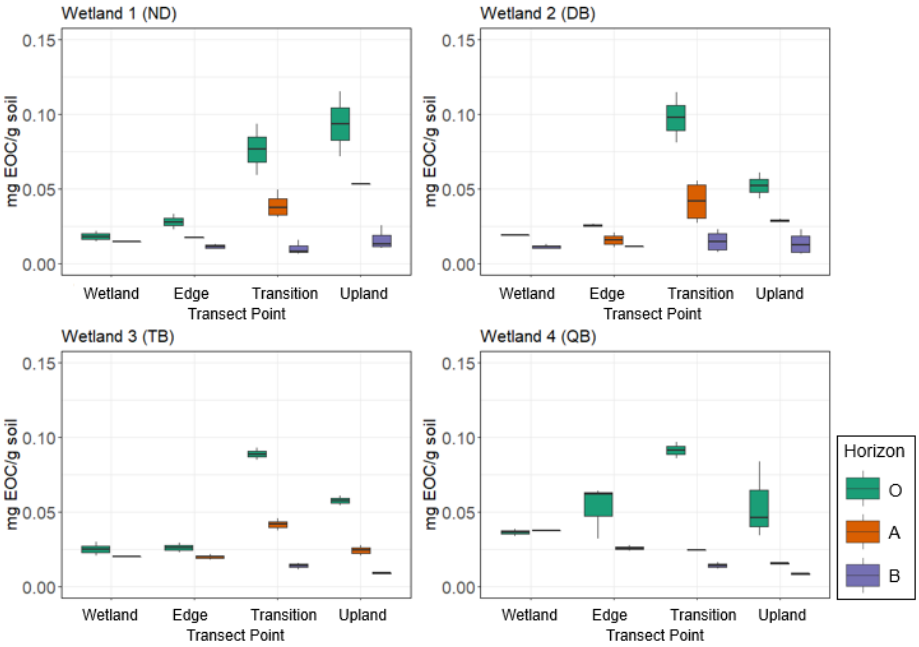

Figure A.9: EOC values for both sampling campaigns separated by wetland site.

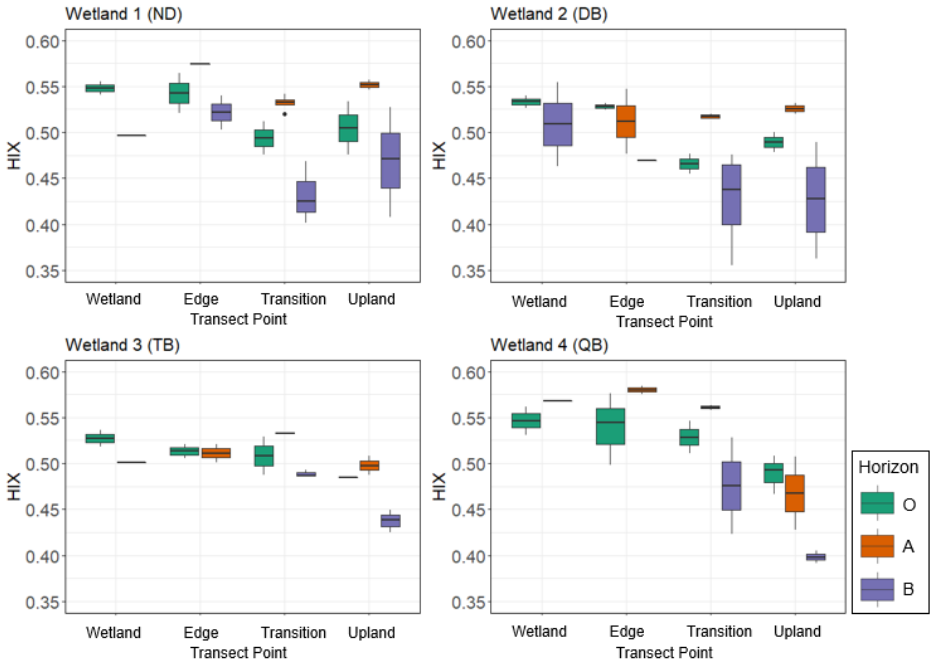

Figure A.10: HIX values for both sampling campaigns separated by wetland site.

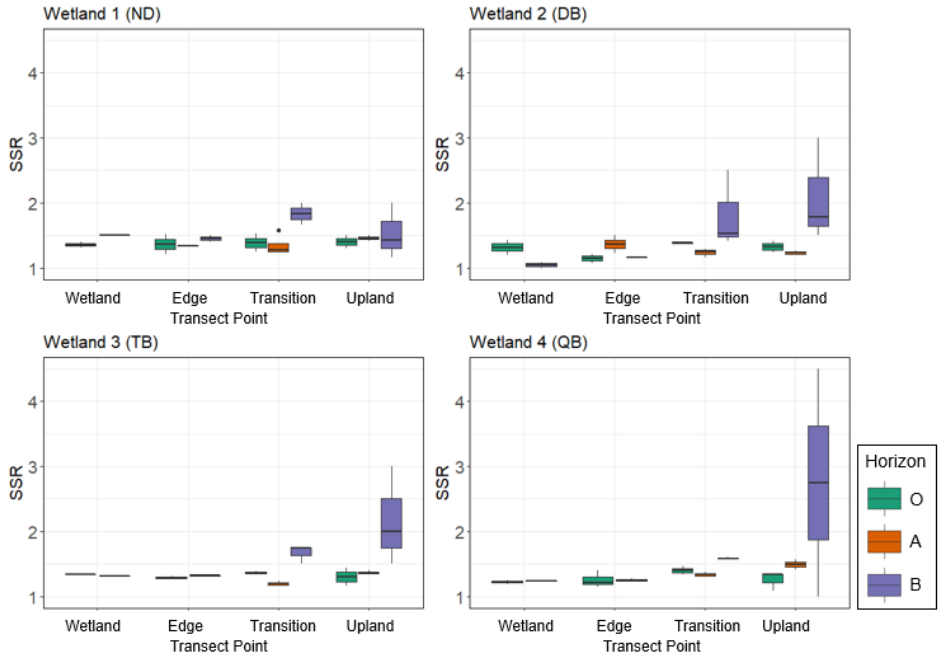

Figure A.11: SSR values for both sampling campaigns separated by wetland site.

Appendix B

Additional Results

This section contains additional results that were not included in the main thesis text.

B.1 Extractable Nitrogen Results

Extractable Total Dissolved Nitrogen (ETDN) and Nitrate (ENO_3) were measured in addition to Extractable Organic Carbon (EOC). ETDN results mirrored EOC values where O horizons had highest observed values and values then decreased with increasing soil depth (Figure B.1). ENO_3 values varied widely between the two sampling campaigns. In spring, values in the Wetland and Edge O and A horizons saw a larger range in values. In autumn, most soil horizons along the transect were below instrument detection and B horizons had the larger range in values. Autumn may see lower ENO_3 values because NO_3 is consumed as an alternate electron acceptor when oxygen is depleted during long-term saturated conditions and because microbial activity is elevated during warmer summer months.

B.2 Channel and Forested Flat

Extractions were performed on soil samples from a Channel and a Forested Flat, in addition to the four wetland sites, to determine if there was a difference in ESOM quantity and quality in-between and "downstream" of GIWs. The Channel connects Wetland 4 to an

adjacent emergent GIW during seasonal high water levels but will dry out during summer dry periods. Forested Flats are low lying forested areas in-between GIWs that are inundated with shallow water levels ($\approx$ 0.3 m) throughout a typical water year. The Forested Flat sampled was located near the outlet of Wetland 4's catchment. Channel and Forested Flat samples were sampled to a depth of approximately 30 cm and sampling occurred with standing water present. Both the Channel and Forested Flat had EOC values similar to the Wetland and Edge soil horizons where saturated conditions result in lower ESOM values (Figure B.2 Panel A). The other fluorescence metrics mirrored transect soil results where ESOM from O and A horizons is aromatic and plant-like seen by elevated $SUVA_{254}$ and lower FI values. The B horizon was only captured at the Forested Flat location. Here, the B horizon was less aromatic and had an increased microbial signature, seen by lower $SUVA_{254}$ and elevated FI values (Figure B.2 Panels B and C). These results indicate that soil horizon is a strong control on ESOM quantity and quality in the Channel and Forested Flat, similar to the Upland to Wetland transects. Observations of seasonal saturation at these points would suggest that they represent potential sources of DOM, but further exploration into site hydrology is required at these locations.

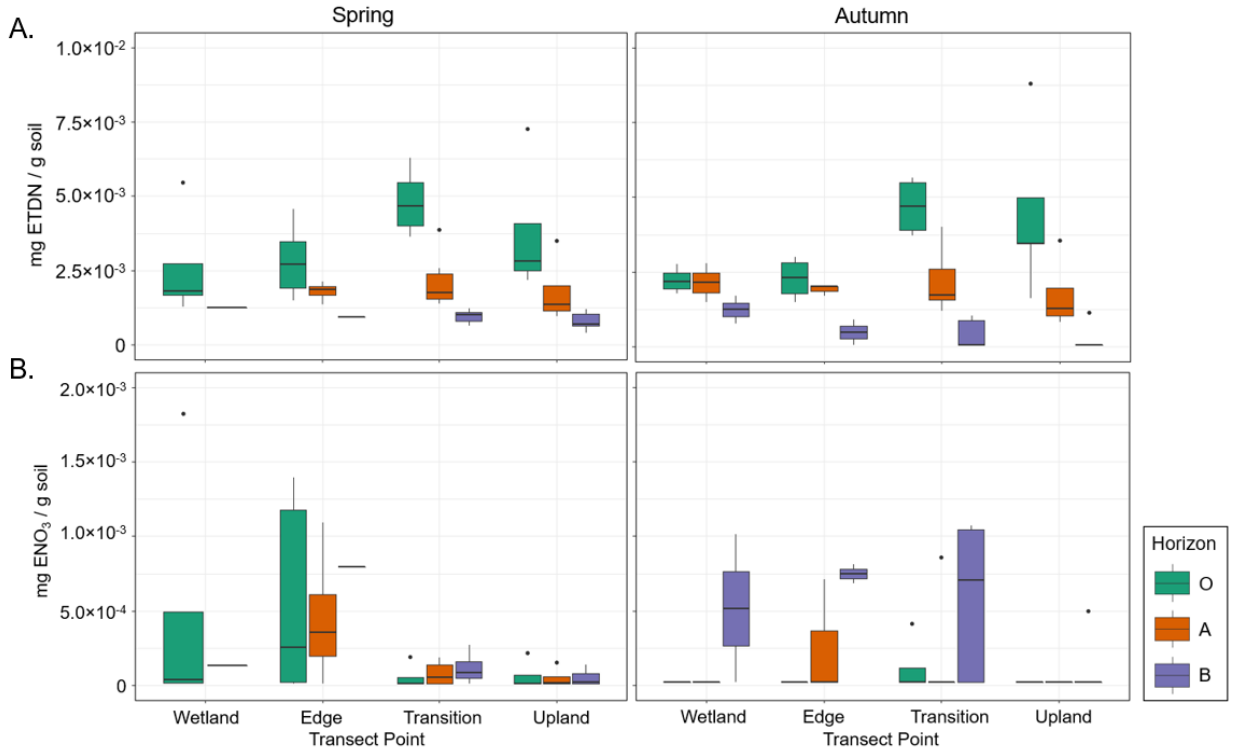

Figure B.1: (A) Extractable Total Dissolved Nitrogen and (B) Extractable Nitrate over the two sampling campaigns across all four sites.

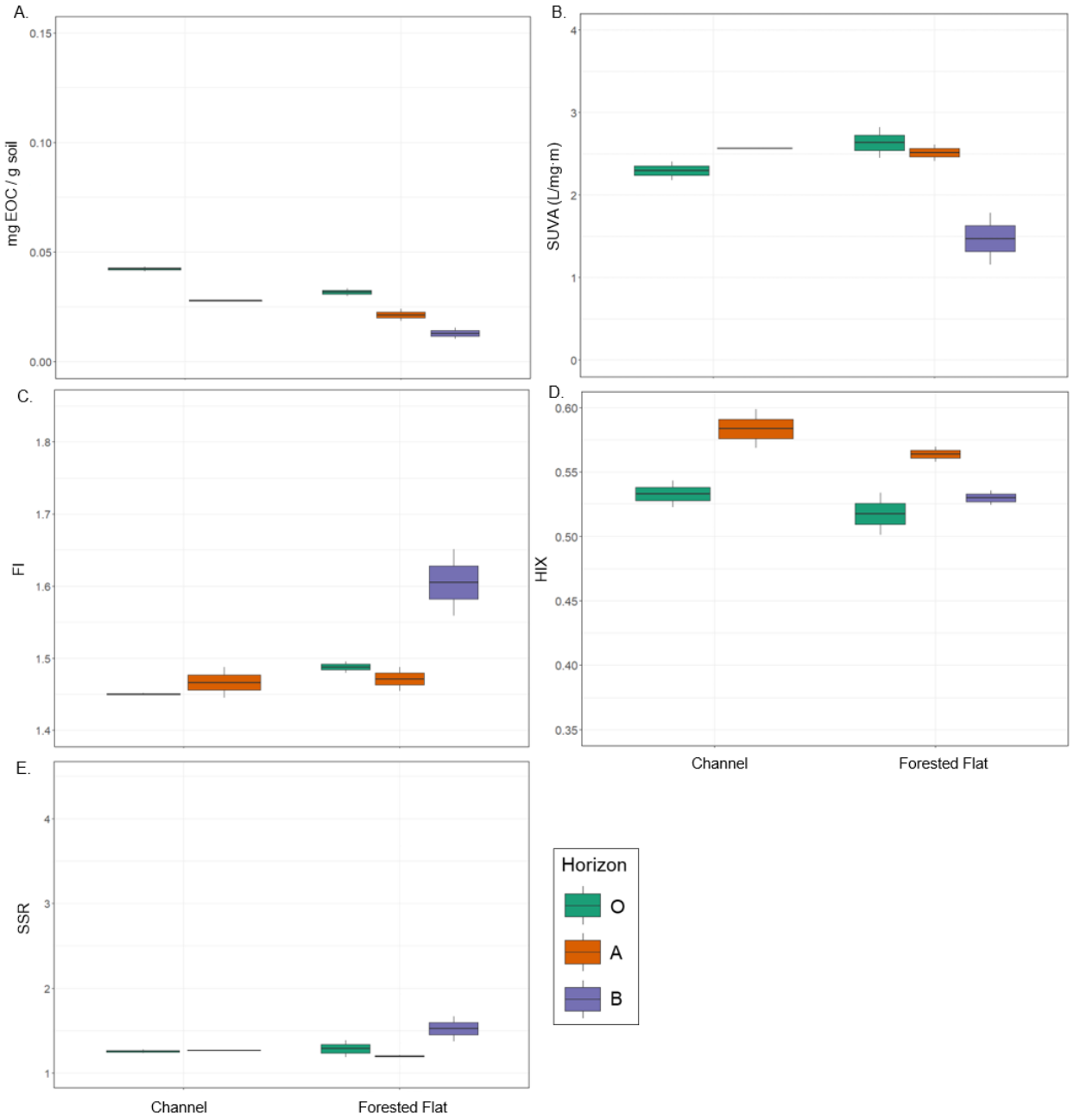

Figure B.2: ESOM results for the Channel and Forested Flat: (A) EOC, (B) $SUVA_{254}$, (C) FI, (D) HIX, and (E) SSR. Results from both sampling campaigns are combined for each metric plot.