THE IMPACT OF SUBMERGED AQUATIC VEGETATION ON NUTRIENT DYNAMICS AND BACTERIAL METABOLISM IN A SOUTHEASTERN RESERVOIR

by

STEPHEN DERRILL SHIVERS

(Under the Direction of Alan Covich and Stephen Opsahl)

ABSTRACT

The concentration and bioavailability of dissolved organic carbon (DOC) can be altered by the autochthonous production of macrophytes, and this alteration can influence microbial processes in aquatic ecosystems. Diel and depth dynamics of water chemistry were studied within a bed of submerged aquatic vegetation (*Hydrilla verticillata*) during a growing season to assess the effects of macrophyte production of DOC on the microbial community of a freshwater reservoir in the southeastern US. This study shows that DOC is produced from submerged aquatic vegetation (SAV) and that a portion of the DOC (monosaccharide) is labile, which alters nutrient cycling within the SAV bed. This study also examines different autotrophs (*Hydrilla, Typha*, *Lyngbya*, and *Potamogeton*) and the utilization of leached carbon from these autotrophs by the microbial community. The findings of this study explain nutrient and carbon dynamics within aquatic ecosystems and the effects that different autotrophs have on nutrient cycling following senescence.

INDEX WORDS: Biogeochemical Cycling, Nutrient Dynamics, Carbon Bioavailability, Lake Seminole, Apalachicola-Chattahoochee-Flint (ACF) Basin, Submerged Aquatic Vegetation, *Hydrilla verticillata*

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CHAPTER 1

INTRODUCTION AND PROJECT OBJECTIVES

Introduction

Although inland freshwater ecosystems comprise a small proportion of the Earth, they make significant contributions to the global carbon cycle (Cole et al. 2007). Inland waters contribute carbon to the atmosphere in the form of CO_2 and methane and can store carbon through burial in the sediment. Inland waters are dynamic sites for carbon cycling and can equal the organic carbon sequestration rates of the ocean floor (Tranvik et al. 2009). In addition to carbon, inland waters play important roles in other biogeochemical cycles, including nitrogen and phosphorus (Harrison et al. 2009). Inland waters can include lakes, rivers, estuaries, wetlands, and reservoirs. Reservoirs, with an estimated global surface area of 1.5 million km^2, could play a crucial role in global biogeochemical cycling (St. Louis et al. 2000). Because reservoirs, particularly those behind large dams, are predicted to become more numerous, their role in biogeochemical cycling could also increase (Downing et al. 2006).

Most reservoirs are constructed as "run of the river" impoundments and are essentially large, slow-moving rivers that receive inputs from the landscape upstream and also from the immediate vicinity. Because of these various inputs, lakes and reservoirs can serve as integrators on a landscape scale (Williamson et al. 2008). By integrating chemical, physical, and biological parameters, lakes and reservoirs indicate changes in these parameters, and thus they can function as sentinels for change due to climate and other factors (Adrian et al. 2009; Schindler 2009). In addition to acting as sentinels for ecosystem change, lakes and reservoirs also help to regulate

changes in climate due to their large influence on carbon cycling (Williamson et al. 2009). Clearly, these influences on biogeochemical cycles require thorough study, especially in the southeastern United States where many reservoirs control river flows.

Biogeochemical Cycling in Freshwater Ecosystems

Freshwater ecosystems are extremely important links in the carbon cycle. Often thought of as "pipes" to transfer carbon to the ocean, recent limnological studies have shown inland waters to also be crucial in the cycling of carbon (Cole et al. 2007; Del Giorgio and Pace 2008; Tranvik et al. 2009). Microbial communities are key components of biogeochemical cycling and carbon cycling in particular, and the regulation of cycling is based in part on microbial metabolism and growth efficiency (Cotner and Biddanda 2002; Del Giorgio and Cole 1998; Paerl and Pinckney 1996). Therefore, the bioavailability of carbon to these communities is critical to their metabolic levels. Labile dissolved organic carbon (DOC) has been consistently shown to increase the metabolism of bacterial communities, and the lability can be affected by the chemical composition of the dissolved organic matter (DOM) (Benner et al. 1986; Sondergaard et al. 1995). The composition and reactivity of DOM can be greatly affected by the source of the DOM, namely whether it is autochthonous or allochthonous (Aitkenhead-Peterson 2003; Bertilsson and Jones Jr. 2003). Autochthonous DOM is generally considered to be more labile, but it alone cannot support the entirety of bacterial production; therefore allochthonous DOM is required to subsidize the needs of the bacterial community even though the community preferentially utilizes autochthonous DOM (Kritzberg et al. 2005; Kritzberg et al. 2004; Pace et al. 2007). In addition to the source of DOM, the microbial community itself can produce large quantities of refractory DOC as it utilizes the more labile carbon for metabolism (Ogawa et al. 2001).

In contrast to stimulation of microbial metabolism by labile DOC, metabolism can be limited if a particular nutrient is in short supply. The limiting nutrient can vary depending on the ecosystem and different studies have yielded different results. Phosphorus has been identified as the sole limiting nutrient (Smith and Prairie 2004) or co-limiting with organic C (Carlsson and Caron 2001), and organic C has been the sole limiting nutrient (Benner et al. 1995). The ratio of DOC: nutrients can also affect microbial metabolism within lakes (Cimbleris and Kalff 1998). Therefore, the mechanisms of nutrient limitation are defined by the environmental context of specific locations, but the general properties are broadly applicable.

In addition to carbon cycling, inland waters are also important sites for nitrogen cycling. Assimilation of nitrogen compounds by organisms and denitrification are two ways in which nitrogen is removed from ecosystems (Burgin and Hamilton 2007). Denitrification converts NO_3 to N_2, is mediated by microbial communities, and removes more N than is produced through N fixation in most aquatic ecosystems (Seitzinger 1988). Because of the role of microbial communities, an increase in C bioavailability can increase microbial metabolism, which can result in increased denitrification rates (Sobczak et al. 2003). Since low O_2 concentrations are required for denitrification to occur, and this process usually occurs in anoxic waters near the sediment, benthic zones are extremely important for nutrient cycling (Carmouze et al. 1998; Houser et al. 2003; Vadeboncoeur et al. 2002).

Impacts of SAV on Physical and Chemical Characteristics of Freshwater Ecosystems

Submerged aquatic vegetation (SAV) is often rooted in the sediment, and because it extends into the water column, these plants link the benthic and pelagic zones and also directly contribute to biogeochemical cycling. SAV has a range of diverse effects on processes that occur within aquatic ecosystems. The impact of SAV can extend from providing cover from

predators to enhancing the food base and improving the reproductive potential of top predators (Brown and Maceina 2002). SAV also competes with phytoplankton by taking up and storing nutrients and affects the turbidity levels of the water by altering water flow rates (Dennison et al. 1993; Havens 2003; Madsen et al. 2001). Two areas affected by SAV are productivity and biogeochemical cycling, the result of which can be seen as changes in the physical and chemical parameters of water quality, such as DO, pH, dissolved organic carbon concentrations, and nutrient concentrations (Carpenter and Lodge 1986).

SAV can cause large changes in the physical environment. An example of these changes is dissolved oxygen (DO) dynamics. Due to high levels of photosynthesis, SAV can release large quantities of O_2 into the water effectively raising the DO concentration. Conversely, due to decomposition that consumes O_2, these O_2 concentrations can be extremely low near the sediment, especially during seasonal die offs (Caraco et al. 2006). SAV can also greatly impact pH levels in the water column. Photosynthetic activity raises pH levels because photosynthesis depletes CO_2, which results in diel changes in pH corresponding to periods of primary production (Van et al. 1976).

SAV can affect nutrient cycling while alive and upon senescence. While living, SAV takes up nutrients to support growth and plant metabolism, thus reducing nutrient concentrations in the water. SAV also releases labile DOC during photosynthesis by cellular exudation (Demarty and Prairie 2009; Penhale and Smith Jr 1977; Wetzel 1969). This labile exudate can cause an increase in bacterial metabolism (Ziegler and Benner 1999). Upon senescence, nutrients are released into the water rapidly during the first 24 hours due to leaching (Webster and Benfield 1986). Afterwards, nutrient release continues at a pace dictated by the chemical composition of the plant (Godshalk and Wetzel 1978). The chemical composition also

determines how quickly DOC can be utilized by the microbial community (Moran and Hodson 1989). A more labile carbon source can be utilized at a faster rate than a more recalcitrant source. Previous studies have shown that a large percentage of carbohydrates are released as DOM, and these carbohydrates could be taken up preferentially by the microbial community (Opsahl and Benner 1999). This relationship could be one reason that Stanley et al. (2003) found macrophytes to influence bacterial production. Additionally, dissolved constituents can result in higher bacterial growth efficiencies, which can lead to more carbon being conserved in the food web (Findlay et al. 1986). Overall, the uptake of nutrients to support photosynthesis and the differential leaching rates and availability of nutrients during senescence can indirectly affect aquatic food webs (Huss and Wehr 2004).

SAV also directly and indirectly affects nitrogen cycling (Flindt et al. 1999). SAV takes up nitrogen directly from the water and sediment to support growth. The bacterial community present within SAV beds can also remove nitrogen through denitrification. Caffrey and Kemp (1992) demonstrated that 75% of nitrogen lost in estuarine sediments was through denitrification while 25 % was through plant uptake. SAV can also affect nitrogen cycling through carbon release. Labile carbon has been shown to lower nitrification rates in streams. These lower rates are thought to be due to heterotrophic bacteria being stimulated by the labile carbon and then outcompeting nitrifiers (Strauss and Lamberti 2002). Indirect effects, such as these, can have larger effects than direct effects (plant uptake) (Hooper and Vitousek 1998). The result of these processes is that SAV can be overall nutrient sinks, especially in shallow lakes, but can also be seasonal nutrient sources (Kufel and Kufel 2002).

In addition to local changes, the impacts of SAV can result in whole lake effects. Beds of SAV are hotspots for labile C production and nitrogen uptake/removal, and these rates are

highest during periods of increased primary production. Because SAV, such as *Hydrilla*, is capable of covering large areas, these hot spots of rapid production can be extended into hot zones, which can significantly reduce NO_3 concentrations at the reservoir scale (McClain et al. 2003). The release of nutrients after senescence can result in large seasonal nutrient differences. These nutrients are labile and, when exported from SAV beds to the pelagic zone, can affect the plankton and bacterial communities, and changes to these communities can affect higher trophic levels (Carpenter 1980; Carpenter 1981). These effects can result in the food web becoming more microbial-based and less algae-based (Porter et al. 1996). Indeed, vegetation has been shown to exert great control over food webs because of primary production (Power 1992). SAV can also greatly affect nutrient concentrations on the whole lake scale by providing shelter for zooplankton. Jeppeson et al. (1997) demonstrated diel patterns of cladocerans using SAV as protection from predatory fish. The protection by SAV resulted in larger populations of cladocerans, which lowered phytoplankton levels and helped to maintain a stable, clear-water state.

Description and Characteristics of Hydrilla verticillata

Submerged aquatic vegetation is a key component of biogeochemical cycling in lakes. These effects on cycling are relevant within Lake Seminole due to the presence of *Hydrilla verticillata* (hydrilla). Hydrilla is a fully submerged plant, primarily native to Asia, which was first discovered in the United States in southern Florida in 1960 (Blackburn et al. 1969). Hydrilla is most commonly found in shallow water but can occur in deeper water (up to 7 m) and can grow in a variety of water conditions ranging from oligotrophic to eutrophic (Cook and Luond 1982; Gu 2006). Like other aquatic plants, hydrilla growth is dependent upon sediment composition and nutrient availability, but the ability of hydrilla to utilize sediment sources of

nutrients helps to reduce nutrient limitation (Barko and Smart 1986; Barko et al. 1988; Bianchini et al. 2010). Hydrilla also decomposes at a fast rate, which can lead to greater seasonal fluxes in nutrients (Battle and Mihuc 2000).

Hydrilla is an adaptable plant that can utilize both free CO_2 and HCO_3^- and can also begin photosynthesis under lower light conditions than other submerged plants (Langeland 1996). But, hydrilla still needs light for photosynthesis and plant biomass is lower under conditions, such as high suspended solids, which reduce light penetration (Havens 2003). Hydrilla plants also have variable CO_2 compensation points that result in different metabolisms (CAM or C_4) depending on environmental conditions (Holaday and Bowes 1980). These adaptations make hydrilla a superior competitor, which may result in the displacement of native vegetation (Wang et al. 2008). As a result of this displacement, the community composition, nutrient cycling, and water flow patterns could be affected on an ecosystem level (Vitousek 1990).

Hydrilla is also efficient during reproduction and is capable of high levels of propagation (Langeland 1996). Reproduction is accomplished primarily by vegetative fragmentation and turion/tuber production, with seed production being less important within a single lake but significant for long-distance dispersal and long-term adaptation. Due to these reproductive strategies, hydrilla can quickly dominate aquatic ecosystems. For example, since hydrilla was introduced into Lake Seminole in 1967, coverage has ranged from less than 40% to greater than 70% of the lake's surface area depending on annual production and control measures (Maceina and Slipke 2004). In addition to being a nuisance because of spatial coverage, hydrilla can also negatively affect bird populations that are susceptible to avian vacuolar myelinopathy (AVM) (Birrenkott et al. 2004). A novel cyanobacterium that is associated with hydrilla has been shown to cause AVM in waterfowl and raptors (Wilde et al. 2005; Wiley et al. 2009). Although Lake

Seminole is currently AVM negative, other reservoirs in the southeastern United States are positive and have experienced avian deaths due to AVM.

The Importance of Lake Seminole and the ACF Basin

The Apalachicola-Chattahoochee-Flint (ACF) Basin extends from the north Georgia mountains to the Gulf of Mexico. Lake Seminole is a 15,216-hectare impoundment located in the karst (limestone) terrain of southwestern Georgia within the ACF Basin (Torak et al. 2006). Lake Seminole is a run-of-the-river reservoir whose primary functions are to aid in navigation and provide hydroelectric power. The mean depth of Lake Seminole is 3 m and the maximum depth is 10.7 m (Sammons and Maceina 2005). The major inflows into Lake Seminole are the Chattahoochee River, Flint River, and Spring Creek. These tributaries drain a combined land area of 46,141 square kilometers. Groundwater also contributes a substantial component of flow into the lake (Torak et al. 2006). The Jim Woodruff Lock and Dam regulates discharge from the lake. The single outflow is the Apalachicola River, which flows south eventually emptying into the Gulf of Mexico at the Apalachicola Bay.

The influence of hydrilla within Lake Seminole and the position of the lake within the ACF Basin could potentially result in regional consequences. The ACF Basin has been the focus of a series of legal disputes among Alabama, Georgia, and Florida since 1989 (Carter et al. 2008). These disputes relate to the need for more water storage capacity in Lake Lanier to support the growing population of metropolitan Atlanta relative to the need for higher flow volumes downstream to protect native species of freshwater mussels and floodplain dynamics. Fisheries (oyster, freshwater, and marine) in Apalachicola Bay are dependent upon sufficient freshwater flow to maintain the salinity levels required for the survival of aquatic life (Corn et al. 2008). Additionally, four threatened species, Gulf Sturgeon (*Acipenser oxyrinchus*), fat

threeridge mussels (*Amblema neislerii*), Chipola slabshell mussels (*Elliptio chipolaensis*) and purple bankclimber mussels (*Elliptoideus sloatianus*), are found in the ACF basin and are dependent upon sufficient water flow. Frequent drought in the southeast has further intensified these tri-state disputes over water (Carter et al. 2008). Some current climate change models predict that future conditions in the southeastern US will be warmer and drier with a higher frequency of extreme weather events (Diffenbaugh et al. 2005; Dore 2005). If the model predictions are correct, changes in climate could further exacerbate the water problems currently faced in the southeast. Because Lake Seminole is the last impoundment before the Apalachicola River flows into the Gulf of Mexico, it is imperative that factors (e.g. *Hydrilla*) which affect carbon and other nutrient cycling be thoroughly studied within the lake in the context of changing inflows and residence times.

Project Objectives

The goal of this project is to study the effects of SAV on biogeochemical cycling within Lake Seminole. A previous study identified the source/sink dynamics of Lake Seminole with respect to carbon and nutrient concentrations (McEntire 2009). Because of the prevalence of hydrilla on Lake Seminole, this project is designed to study the effects that SAV can have on the physical and chemical characteristics of water column DOC and nutrients. This study seeks to achieve the following objectives:

Objective 1: To determine the effects of SAV (*Hydrilla verticillata*) on nutrient and dissolved organic carbon concentrations in the water column.

Objective 2*:* To evaluate the bioavailability of dissolved organic carbon from *Hydrilla verticillata* to microbial communities.

Objective 3: To evaluate the bioavailability of dissolved organic carbon from four different primary producers and to relate the differences in bioavailability to carbohydrate concentrations.

Objectives 1 and 2 required a field study consisting of intensive 24-hour sessions followed by extensive laboratory analysis. Objective 3 was primarily a laboratory study using living biomass collected from Lake Seminole.

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CHAPTER 2

THE DIEL AND SEASONAL EFFECTS OF SUBMERGED AQUATIC VEGETATION ON NUTRIENT DYNAMICS AND ORGANIC CARBON BIOAVAILABILITY IN A SOUTHEASTERN RESERVOIR[1]

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Abstract

The concentration and bioavailability of dissolved organic carbon (DOC) can be altered by the autochthonous production of macrophytes, and this alteration can influence microbial processes in aquatic ecosystems. In order to assess the effects of macrophyte production of DOC on the microbial community of a freshwater reservoir, diel and depth dynamics of water chemistry were studied within a bed of submerged aquatic vegetation (*Hydrilla verticillata*) during a growing season. DOC concentrations exhibited the greatest variability among depths during August (mean diurnal surface concentration = 3.91 mg/L and above the sediment concentration = 3.05 mg/L) and September (mean surface concentration = 2.88 mg/L and benthic concentration = 4.18 mg/L). Diurnal carbon bioavailability was higher near the sediment during September based on higher O_2 consumption rates observed during the day (1.09 µmol/h) than at night (0.52 µmol/h). Monosaccharide concentrations also followed the same pattern as DOC and could explain the increased carbon bioavailability. Therefore, it is likely that an increase in bioavailable carbon led to an increase in microbial metabolism. NO_3 concentrations were consistently lower during the growing season near the sediment (mean = 189 µg/L) compared to the surface (mean = 705 µg/L) or the intermediate depth (mean = 838 µg/L). These lower NO_3 concentrations indicate that nitrate was being removed from the system either through assimilation or denitrification. These findings show that DOC is produced from SAV, and that a portion of the DOC (monosaccharide) is labile, which can result in alteration of nutrient cycling within the SAV bed.

Introduction

Although inland freshwater ecosystems comprise a small proportion of the Earth, they make significant contributions to the global carbon cycle (Cole et al. 2007). Inland waters contribute carbon to the atmosphere in the form of CO_2 and methane, can store carbon through burial in the sediment, and can help to regulate biogeochemical cycling on a global scale (Tranvik et al. 2009). Because of the coupling of inland waters to terrestrial ecosystems, inland waters can serve as integrators on a landscape scale and can function as sentinels for a changing climate (Schindler 2009; Williamson et al. 2008). Reservoirs, having an estimated surface area as high as 1.25 million km^2, are a critical component of inland waters (St. Louis et al. 2000). Due to their increasing numbers, the contribution of reservoirs to nutrient cycling will become more significant in the near future (Downing et al. 2006). The inputs of carbon to reservoirs include not only riverine, groundwater, and atmospheric sources, but also autochthonous sources, which includes submerged aquatic vegetation (SAV)(Aitkenhead-Peterson 2003; Bertilsson and Jones Jr. 2003). SAV can alter the productivity and biogeochemical cycling within a reservoir; these effects can result in changes in the physical and chemical parameters of water quality, such as DO, pH, and dissolved organic carbon (DOC) concentration (Carpenter and Lodge 1986). SAV can: i). be a major source of nutrients, energy, and DOC within lentic ecosystems (Carpenter 1980), ii). compete with phytoplankton for nutrients (Van Donk and Van De Bund 2002), and iii). be a sink for nutrients within an ecosystem (Carpenter and Lodge 1986; Dierberg et al. 2002; Takamura et al. 2003). Overall, these relationships can result in SAV being a controlling factor within aquatic ecosystems (Jeppesen et al. 1997).

In addition to SAV affecting aquatic ecosystems directly, SAV can also indirectly affect these ecosystems by providing labile carbon or nutrients to microbial communities (Huss and

Wehr 2004). The availability of nutrients and oxygen to these communities can affect their metabolism. Because microbial communities are the primary mediators for biogeochemical cycling, this essential cycling can be altered (Paerl and Pinckney 1996; Ziegler and Benner 2000). These alterations are manifested in changes in the nitrogen cycle, specifically nitrification and denitrification (Sobczak et al. 2003; Strauss and Lamberti 2002).

The purpose of this study was to evaluate the impact both spatially and temporally that SAV exerts upon carbon dynamics and also to assess the bioavailability of DOC from SAV beds. Carbohydrate concentrations were measured because they comprise a labile component of DOC. The effects of labile DOC upon the microbial community and the resulting implications were also investigated by measuring O_2 consumption during whole water incubations. Finally, nutrient limitation of the microbial communities within SAV beds was also assessed with a series of nutrient amendment experiments.

Methods

Site Description

The Apalachicola-Chattahoochee-Flint (ACF) Basin extends from the north Georgia mountains to the Gulf of Mexico (Figure 2.1). Lake Seminole is a 15,216-hectare impoundment located in southwestern Georgia within the ACF Basin (Torak et al. 2006). Lake Seminole is a run-of-the-river reservoir whose primary functions are to aid in navigation and provide hydroelectric power. The mean depth of Lake Seminole is 3 m and the maximum depth is 10.7 m (Sammons et al. 2005). The major inflows into Lake Seminole are the Chattahoochee River, Flint River, and Spring Creek. These tributaries drain a combined land area of 46,141 square kilometers. Groundwater also contributes a substantial component of flow into the lake (Torak et al. 2006). This study was conducted in Spring Creek (30° 47’40” N; 84°47’8” W), which is

characterized by low DOC, low PO_4, and high NO_3 concentrations (McEntire 2009). The Jim Woodruff Lock and Dam regulates discharge from the lake. The single outflow is the Apalachicola River, which flows south eventually emptying into the Gulf of Mexico at Apalachicola Bay.

One important feature of the lake is the proliferation of *Hydrilla verticillata* since 1967. Hydrilla is a fully submerged plant, native to Asia, which was first discovered in the United States in southern Florida in 1960 (Blackburn et al. 1969). Hydrilla is most commonly found in shallow water but can occur in deeper water (up to 7 m) and can grow in a variety of water conditions ranging from oligotrophic to eutrophic (Cook and Luond 1982; Gu 2006). Hydrilla is a highly adaptable plant that can utilize both free CO_2 and HCO_3^- and can also begin photosynthesis under lower light conditions than other submerged plants (Langeland 1996). These adaptations make hydrilla a superior competitor, which may result in the displacement of native vegetation (Wang et al. 2008). Hydrilla is also efficient during reproduction and is capable of high levels of propagation (Langeland 1996). For example, since hydrilla was introduced into Lake Seminole in 1967, coverage has ranged from less than 40% to greater than 70% of the lake's surface area depending on annual production and control measures (Maceina and Slipke 2004).

Physical/Chemical Measurements

All measurements were taken over a 24-hour time period once per month during the months of August, September, October, and December 2009. Temperature, dissolved oxygen (DO), pH, conductivity, and salinity were measured in situ using a Hydrolab Quanta System (Hach Laboratories). These measurements were taken every 3 hours at 3 depths (surface, intermediate, and near the sediment). Water samples were also collected at the same depths and

times using an ISCO pump. These samples were stored in 1 L Nalgene polycarbonate bottles and immediately placed on ice until returning to the lab. The samples were filtered through pre-ashed 0.7 µm glass fiber filters within 72 hours of collection and stored appropriately (Monosaccharides, NO_3, PO_4, TN, and TP below 0 °C; NH_4 and DOC between 0-5 °C) until analysis. These samples were later analyzed for dissolved organic carbon (DOC), monosaccharides, NO_3, NH_4, PO_4, TN, and TP. DOC was measured using a Shimadzu TOC-V total carbon analyzer. Dissolved monosaccharides were measured using the 2,4,6-Tripyridyl-s-triazine (TPTZ) colorimetric method of Myklestad modified by Hung (Hung et al. 2001). Absorbance measurements were made using a Shimadzu UV-2101 spectrophotometer. NO_3, NH_4, and PO_4 concentrations were determined using a Lachat QuikChem 8500 and the corresponding appropriate method. TN and TP were also analyzed using the Lachat QuikChem, but were first digested using a microwave persulfate digestion (Johnes and Heathwaite 1992).

Carbon Utilization Bioassays

Water samples were collected during the same months and at the same depths as previously described, however the samples were collected every 6 hours over a 24-hour period. Water was collected using a peristaltic pump to collect the water from a specific depth and was filtered during collection with an inline 0.7 µm pre-ashed glass fiber filter. The sample water was bubbled using an aquarium air stone in order to alleviate anoxia and equilibrate O_2 saturation among depths. Eighteen 60 ml biological oxygen demand (BOD) bottles were filled with the filtered water (Figure 2.2). Four bottles were immediately fixed using NaI/NaOH and $MnSO_4$ solutions to establish time 0. Nutrient amendments (3.21 g/L NH_4Cl, 10.8 g/L $C_6H_{12}O_6$, 16.1 g/L $Na_2HPO_4 \cdot 5H_20$, and 5.1 g/L $NaNO_3$) were added to two bottles for each treatment (Opsahl 2005). Six bottles remained unamended to provide six hour and 24 hour time points as

controls. All bottles were incubated under dark conditions at a constant temperature for 24 hours at the water temperature of the initial collection. Four bottles were fixed after 6 hours and the two unamended and eight amended BOD were fixed after 24 hours. Dissolved oxygen concentrations were determined through titration utilizing the Winkler method and a Mettler Toledo DL50 titrator (Pomeroy et al. 1994). Because the respiratory quotient of the microbial community was assumed to be 1 mole of carbon:1 mole of oxygen, we were able to estimate the carbon metabolism based on the oxygen metabolism (Volkmar and Dahlgren 2006).

Statistical Analysis

Analysis of variance (ANOVA) was used for statistical testing of hypotheses. These tests were two-way ANOVAs; if the p-value for the interaction effect was less than 0.05, then ANOVAs were executed at each of the three depths. Parameter estimates, using the least square means method and correlation coefficients, were also used to investigate interaction effects. All of these statistical analyses were performed using R (R ver. 2.12.0). To evaluate the significance of time and depth on the rates of O_2 consumption, the standard errors of the slopes were calculated using SigmaPlot (SigmaPlot ver. 11.0). These values were then used to distinguish significance among the rates.

Results

Physical Measurements

Temperature varied considerably among depths during August, September, and October, and higher surface temperatures occurred during the day (Figure 2.3). Vertical stratification was greater during August and decreased to isothermal conditions throughout the water column in December. Dissolved oxygen concentrations varied seasonally with greater variation occurring during warmer months (Figure 2.4). Clear diel differences were observed during August,

September, and October for the upper depths (0 m and 1 m). The higher DO concentrations during the day near the top of the hydrilla bed (maximum concentrations for August = 16.8 mg/L, September = 15.6 mg/L, and October = 13.9 mg/L) signify that the water was supersaturated with O_2 and indicate that high levels of primary production were occurring. It is important to note that hydrilla was not topped out during October, but instead was approximately 1 m below the surface. Therefore, the intermediate depth measurements (1 m) showed the maximum DO concentration. Benthic DO concentrations were consistently low during these months (mean DO for August = 2.31 mg/L, September = 0.90 mg/L, and October = 1.6 mg/L) indicating that decomposition by heterotrophic microorganisms was consuming available O_2. DO concentrations were stable during December and showed no diel or depth changes.

Observed pH levels varied among depths and exhibited diel differences between August and October (Figure 2.5). The pH values increased during the day near the surface (August max = 9.83, September max = 9.28, and October max = 8.61). These increases were due to high levels of photosynthesis by hydrilla that removed CO_2 and HCO_3^- from the water column. Benthic pH levels remained fairly constant during the sampling periods and between the months of August – October (August mean = 7.46, September mean = 7.61, October mean = 7.69). There was no diel or depth variation during the month of December.

Chemical Measurements

Dissolved organic carbon concentrations exhibited distinctly different dynamics for each month (Figure 2.6). During August, clear diel patterns and significant differences among the depths were observed (ANOVA, $p<0.01$). Surface DOC concentrations increased sharply during the day (from 3.77 mg/L to 4.28 mg/L), while benthic concentrations remained low (mean = 2.86 mg/L), and the middle depth was intermediate (mean = 3.33). DOC concentrations were

distinctly different in September with concentrations above the sediment being significantly higher than the other depths (mean = 4.18 mg/L)(ANOVA, $p<0.01$). DOC concentrations at the surface (mean = 2.88 mg/L) and the intermediate depth (mean = 2.76 mg/L) showed little diel change during September. DOC concentrations were stable among depths and exhibited little diel change during October and December. Monosaccharide concentrations, a component of the DOC measurements, closely followed the DOC patterns (Figure 2.7). Significant diel and depth differences were observed during August with higher concentrations near the surface and lower concentrations near the sediment during the day (ANOVA, $p<0.01$). During September, benthic monosaccharide concentrations were significantly higher than the other depths (ANOVA, $p<0.01$). Unlike DOC, monosaccharide concentrations remained constant with depth during October (mean = 0.29 mg C/L) and December (mean = 0.33 mg C/L).

NO_3 concentrations were significantly lower near the sediment during August (mean = 77.77 µg/L)(ANOVA, $p<0.01$), September (mean = 280.54 µg/L)(ANOVA, $p<0.01$), and October (mean = 208.75 µg/L)(ANOVA, $p<0.01$) when compared to the surface (monthly means = 421 µg/L, 894.89 µg/L, and 799.59 µg/L, respectively) or the intermediate depth (monthly means = 362.58 µg/L, 1093.13 µg/L, and 1059.63 µg/L, respectively) (Figure 2.8). There were no differences among depths or diel patterns during December. NH_4 concentrations exhibited significant, strong diel patterns near the sediment during August as seen by the large spike of NH_4 during the day (maximum = 640.33 µg/L)(ANOVA, $p<0.01$) (Figure 2.9). There were no significant diel patterns among the surface or intermediate depths during September or October, but NH_4 levels above the sediment were consistently and significantly higher than the other depths (ANOVA, $p<0.01$). In December, there were no depth or diel differences. PO_4 concentrations were near or below the detection limit for all months and all depths.

Carbon Bioavailability

Mean O_2 consumption rates were significantly higher near the sediment (1.17 µmol/h) than the other depths (surface = 0.51 µmol/h and intermediate = 0.64 µmol/h) in September (Figure 2.10). Mean O_2 consumption rates were also higher during the day per depth (0.61, 0.84, and 1.55 µmol/h for 0 m, 1 m, and 1.7 m respectively) than at night (0.42, 0.61, and 0.45 µmol/h). These data indicated that photosynthetic processes were likely influencing carbon bioavailability. The same diel patterns were observed during October, although less strongly, but there was no significant difference among the depths. During December, there were no significant diel or depth differences. O_2 consumption rates were significantly higher with supplemental carbon added (mean = 4.81 µmol/h) for all depths, times, and months than for other treatments (mean = 0.36 µmol/h) (Figure 2.11). This increased consumption of O_2 indicated that the bacterial communities were carbon limited. Interestingly, O_2 consumption rates were significantly higher with phosphate addition at most times and depths during September and October, indicating probable co-limitation (Figure 2.12). It is important to note that the O_2 consumption data for August could not be included due to an experimental problem that was corrected in the later months.

Discussion

SAV Controls on DOC Quantity and Quality

Submerged aquatic vegetation has been shown to have a variety of large effects on various aspects, both physical and chemical, of the surrounding water (Carpenter and Lodge 1986). We found that SAV had large impacts on these aspects over both diel and seasonal timescales. For example, SAV can cause diel and seasonal fluxes of DOC concentrations. We found a 12.2% increase (from 3.6 mg/L to 4.3 mg/L) in DOC concentrations at the surface

during the month of August. These large diel changes are most likely due to extracellular organic carbon excretion by the *Hydrilla* plants during photosynthesis. Diel patterns of increased DO concentrations and pH levels at the surface confirm that photosynthetic activity was very high during this period, and previous studies support the extracellular exudation of DOC from submerged plants (Penhale and Smith Jr 1977; Wetzel 1969). These increased DOC concentrations could have a significant impact on a reservoir-wide scale because some plants, such as *Hydrilla*, are capable of high levels of primary production and can cover large areas of aquatic systems (Langeland 1996), which can translate these local effects to a wider area. We also observed a 19.7% decrease in DOC concentrations during the day from the nighttime mean during August. This decrease is likely due to microbial processes using DOC at a higher rate than it is being produced.

DOC is not only produced during photosynthetic exudation, but also during the decomposition of organic matter at the sediment-water interface (Bertilsson and Jones Jr. 2003). This decomposition can result in fluxes of DOC from the sediment (Figure 2.13). We saw large increases (from 2.81 mg/L to 4.18 mg/L) between the surface and intermediate depths and the above the sediment depth during September. The observed release of DOC near the sediment is likely a result of the decomposition of DOM. This effect is supported by the diurnal increase near the sediment of NH_4, which is produced during remineralization of DOM. These seasonal and diel patterns are in agreement with the results of Ziegler and Benner (1999) regarding DOC and NH_4 fluxes in seagrass beds. During October and December, the lack of diel patterns suggests that the hydrilla plants were not as photosynthetically active as in previous months, and lower DO concentrations and pH levels support this conclusion. This decline supports the idea that hydrilla was driving the patterns observed in August and September. Overall, DOC

concentrations were higher in December (mean = 3.95 mg/L) than October (mean = 2.86 mg/L) and this increase was likely due to an increase in background DOC concentrations, which could result from increased wetland flushing.

In addition to DOC fluxes, this study also found that fluxes of monosaccharides closely followed the same patterns as DOC. For example, the higher surface concentrations in August (max = 0.58 mg C/L) are also most likely a result of exudation from cells during photosynthesis. Additionally, the decrease near the sediment (min = 0.36 mg C/L) is likely due to consumption during decomposition exceeding sediment production. The increased concentrations near the sediment (mean = 0.40 mg C/L), as compared to the other depths (mean = 0.29 mg C/L), during September indicate that there is sediment release of monosaccharides through decomposition. These results are in agreement with previous studies that have shown carbohydrates are released upon decomposition of vascular plant tissue (Opsahl and Benner 1999). The lack of diel and depth changes of monosaccharide concentration during October and December indicate that photosynthesis by hydrilla was driving the fluxes seen in August and September.

The production and release of monosaccharides are important because monosaccharides provide a relatively labile form of carbon that can be readily taken up by microbes. These labile monosaccharides are in contrast to more recalcitrant bulk DOC. Due to the short residence time (avg. = 19 days) of this run-of-the-river reservoir (McEntire 2009), there is likely a large quantity of refractory allochthonous DOC being introduced. Although allochthonous DOC is utilized by the bacterial community, autochthonous DOC, which is more labile, is preferentially used and can support higher bacterial metabolism (Del Giorgio and Pace 2008; Kritzberg et al. 2004).

Overall, the percentage of labile DOC, in the form of monosaccharides, agrees with what Søndergaard and Middelboe reported (14±8%) across a range of lakes (Søndergaard and

Middelboe 1995). The presence of labile DOC could potentially result in higher levels of microbial activity within these beds of macrophytes. This increased activity is consistent with what was observed during this study. In September, oxygen consumption rates were higher near the sediment (1.17 μmol O_2/hr) than at other depths. Because the microbial community uses oxygen during respiration and metabolism, a higher rate of oxygen use, or consumption, indicates a higher rate of microbial metabolism. All other factors being equal, a higher rate of oxygen consumption implies a greater availability of carbon to the microbial community (Opsahl 2005). Therefore, the higher overall oxygen consumption rate near the sediment during September indicates that a more bioavailable form of carbon was present. These higher O_2 consumption rates correlate with higher DOC and monosaccharide concentrations near the sediment at that time and are consistent with the increased bacterial growth rates demonstrated by Hopkinson Jr et al. (1998). Additionally the results show that oxygen consumption rates were almost two-fold higher during the day than at night, which implies that photosynthesis by hydrilla was contributing to the differences in carbon bioavailability. These results are similar to other studies, which have evaluated autochthonous production of carbon (Kritzberg et al. 2005; Stanley et al. 2003). The lack of significant oxygen consumption differences among depths during October and December is also correlated with no significant DOC or monosaccharide differences among depths. Collectively, these observations imply that photosynthesis by hydrilla is driving these changes in carbon bioavailability.

Substrate Controls on Microbial Metabolism in SAV Beds

The availability of carbon is critically important to microbial communities within the reservoir due to the apparent carbon limitation of these communities. In this study, glucose amendments caused oxygen consumption rate to increase substantially at all depths, times, and

months (from 0.78 μmol O_2/hr to 4.81 μmol O_2/hr). The increased rates mean that the metabolism of the microbial community was stimulated and further implies that the community was carbon limited (Opsahl 2005; Vrede et al. 1999). Other studies have not found consistent organic carbon limitation (Carlsson and Caron 2001; Elser et al. 1995; Smith and Prairie 2004). Most of this evidence suggests that carbon is co-limiting with the other nutrient being more strongly limiting than carbon. The fact that these microbial communities are enhanced by additions of labile DOC underscores the importance of localized production of bioavailable constituents such as monosaccharides both in the water column and benthic environments.

There was also evidence that the microbial communities were co-limited by phosphorus. This limitation was seen when the phosphate amendment stimulated microbial metabolism. This stimulation was not as strong an effect as with the carbon amendment and was not present at all times and all depths. A potential reason for this co-limitation is that the background P concentrations were extremely low in the study area. The low P concentrations combined with highly productive, dense hydrilla beds also utilizing P could explain the co-limitation. These results are similar to the results of studies by Chraznowski and Grover and Elser et al., except that both of these studies found P to be more strongly limiting than C (Chrzanowski and Grover 2001; Elser et al. 1995).

Coupling DOC and Nitrogen Cycling in SAV Beds

Nitrogen cycling is rapid and dynamic in shallow systems dominated by SAV. This study found surface NO_3 concentrations to be lower during August (mean = 421 μg/L) than any of the other months. Because August is the peak growing season, the lower NO_3 concentrations could be the result of increased uptake by hydrilla (Barko et al. 1988). This uptake is supported by the diel pattern present during August (daylight mean = 289.3 μg/L and night mean = 500

µg/L), which supports higher rates of NO_3 uptake during higher periods of photosynthesis. Of greater significance is that concentrations of NO_3 near the sediment were extremely low overall (77.8 µg/L), and diel patterns were observed (daytime mean = 18.4 µg/L and night mean = 113.4 µg/L). Combined with appropriate conditions (i.e. low mean DO concentrations = 2.31 mg/L), these data imply that NO_3 is being converted to N_2 through denitrification, and it is being removed from the system. The availability of labile C being produced through photosynthesis and decomposition could be functioning as a driver to increase these rates of denitrification (Boyer et al. 2008). The rise in NO_3 concentrations in the evening could be due to water exchange or diffusion bringing NO_3 into the SAV bed combined with a lack of labile C or rising O_2 conditions reducing denitrification rates. The diel patterns become less clear during September and October, but the concentrations of NO_3 above the sediment remain low (September mean = 280.5 µg/L and October mean = 208.8 µg/L), which indicates that nitrate removal and/or uptake is still occurring within the lake. There are no significant depth or diel NO_3 concentration changes in December. These patterns support the idea that SAV was affecting nitrogen cycling during the growing season.

The autochthonous production of carbon as monosaccharides by SAV provides a labile energy source for the microbial community. Because these microbial communities appear to be carbon limited, the availability of a labile C source can greatly increase their productivity. Due to the extensive coverage of hydrilla, the increased productivity can produce significant regional effects. Previous studies have shown reservoirs to be effective sinks for NO_3 (Harrison et al. 2009; McEntire 2009). This study helps to elucidate the sink dynamics by identifying that submerged aquatic vegetation can have significant direct impacts through NO_3 uptake by the plants; SAV can also indirectly affect nutrient dynamics by stimulating the microbial community

through release of labile C. Considering that Lake Seminole is located below an agricultural region with high fertilizer use, the sink dynamics of the reservoir are potentially significant for areas downstream of the reservoir. In addition to affecting nutrient cycling, the increase in microbial production could have effects on the food web. These effects could include altering the base of the food chain and could extend to both predator and prey. Food web alteration could also affect nutrient cycling, as these effects would eventually return to impact either the SAV or the microbial community.

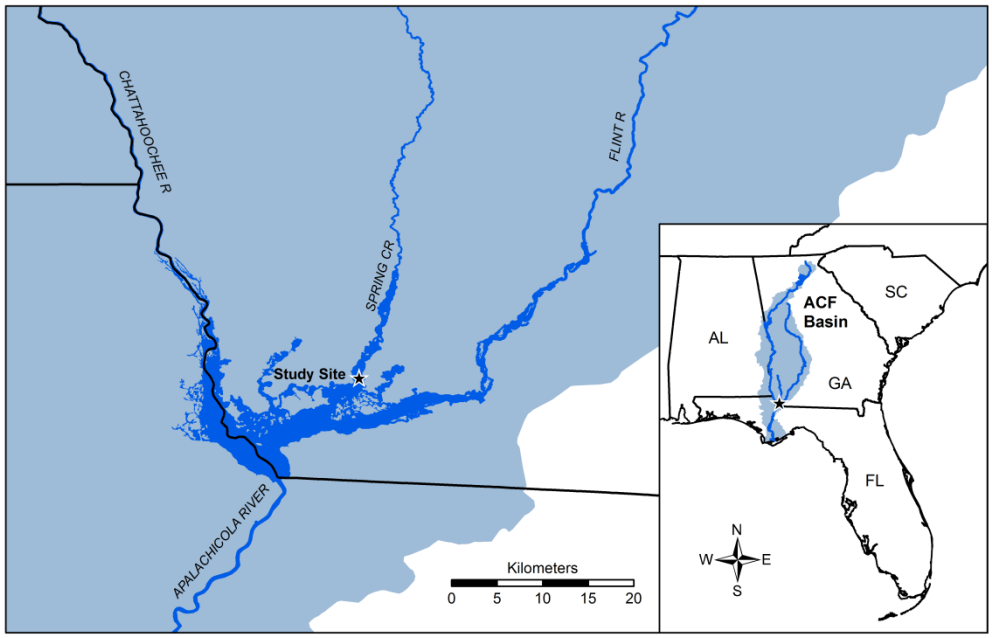

Figure 2.1: The ACF Basin covers land within Georgia, Alabama, and Florida. Lake Seminole is located in the southwest corner of Georgia. The site for this study was located in Spring Creek.

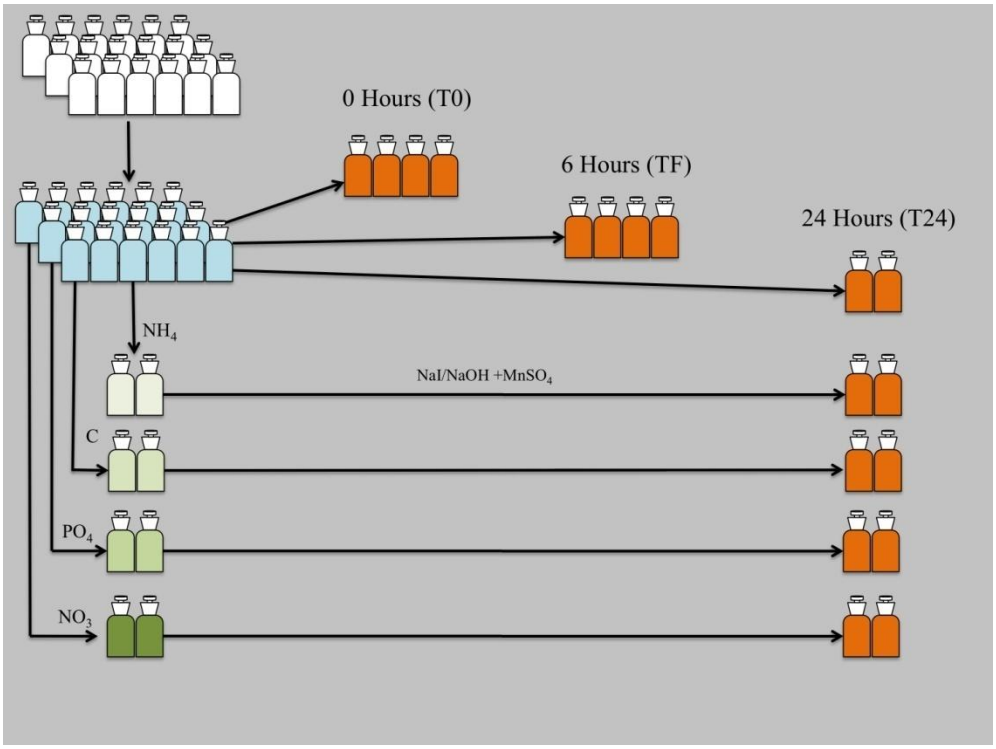

Figure 2.2: A conceptual model describing the BOD bottle experiment including nutrient amendments.

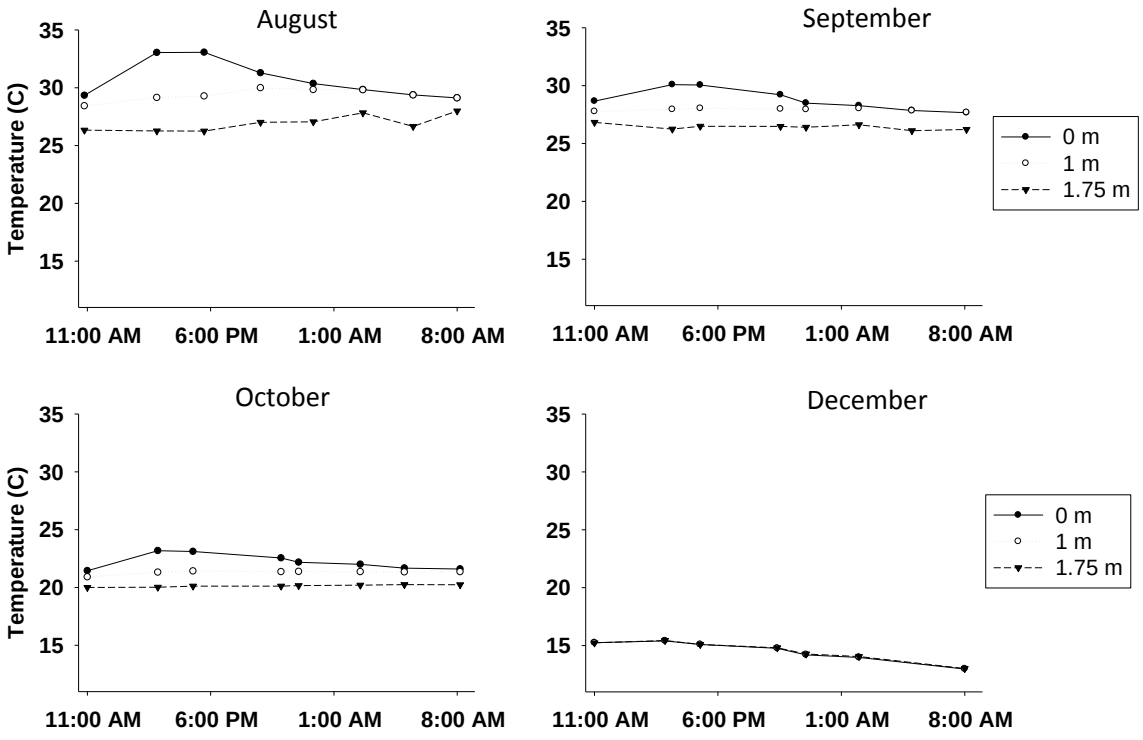

Figure 2.3: Diel temperature changes during the growing season. Greater stratification was observed during the summer months, and no stratification was observed in December.

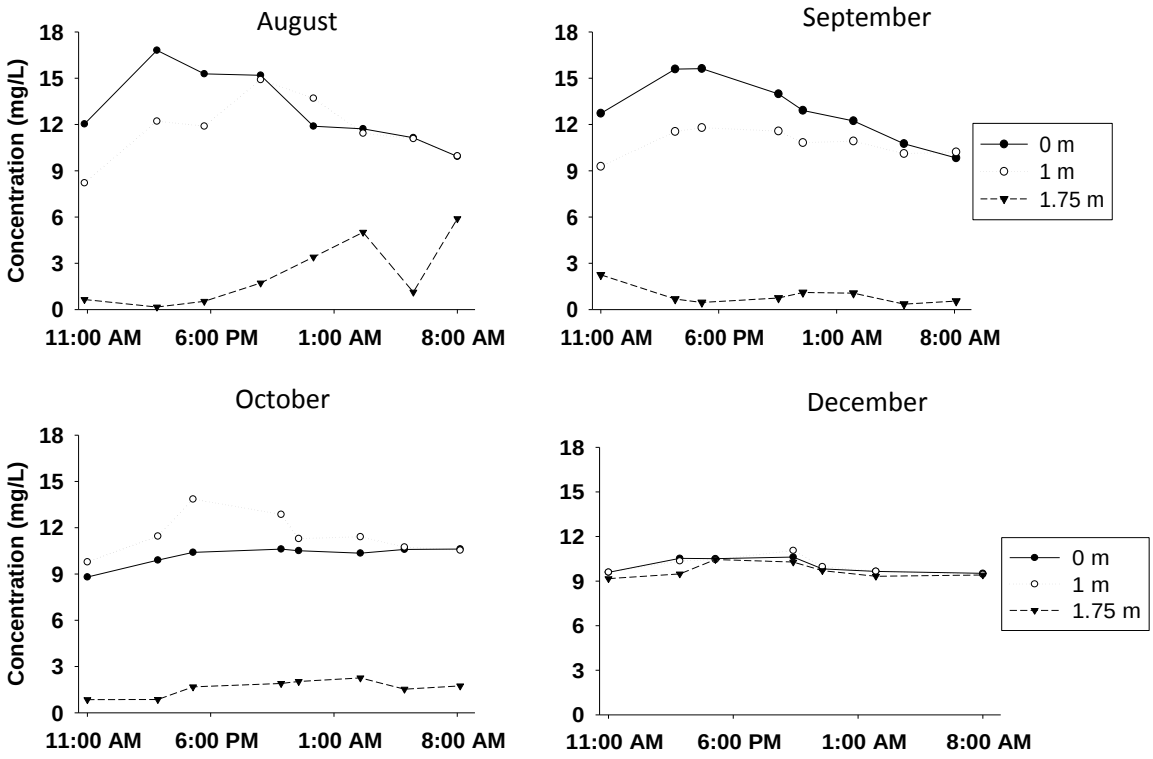

Figure 2.4: Dissolved oxygen exhibited diel and seasonal differences. The surface water was supersaturated during periods of high photosynthetic activity. In contrast, water near the bottom was low (periodically anoxic) during all months except December.

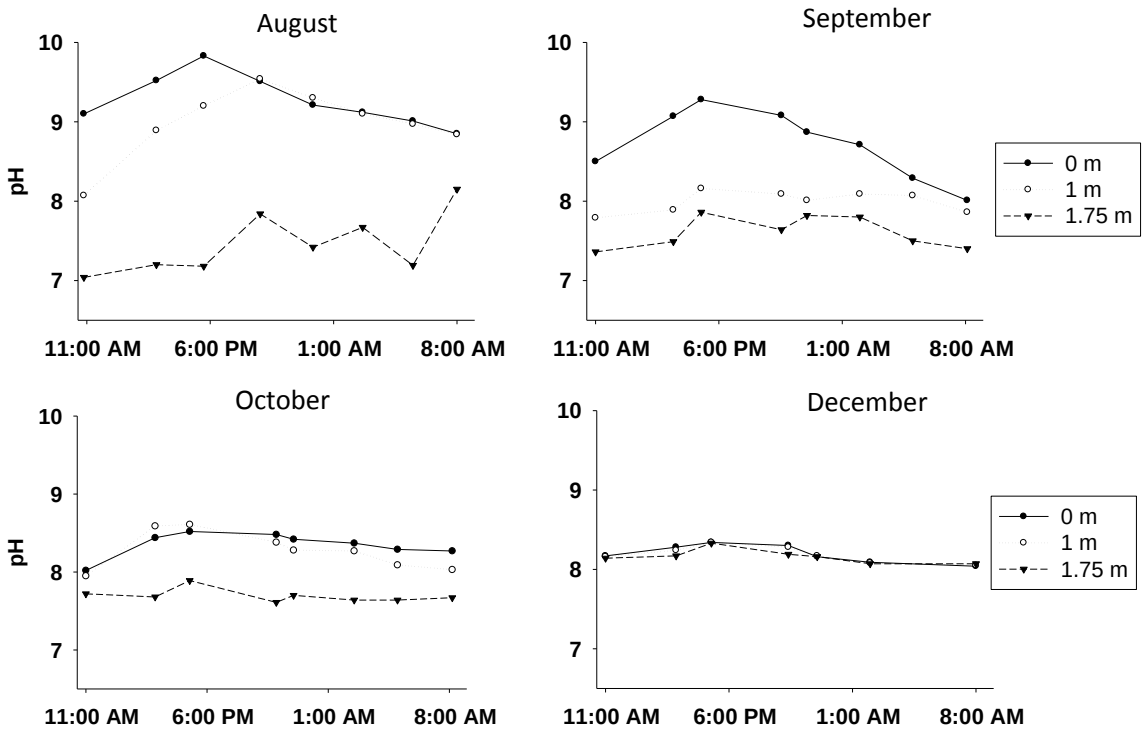

Figure 2.5: Large differences in pH were observed during August and September. These differences were a result of high photosynthetic activity during these months.

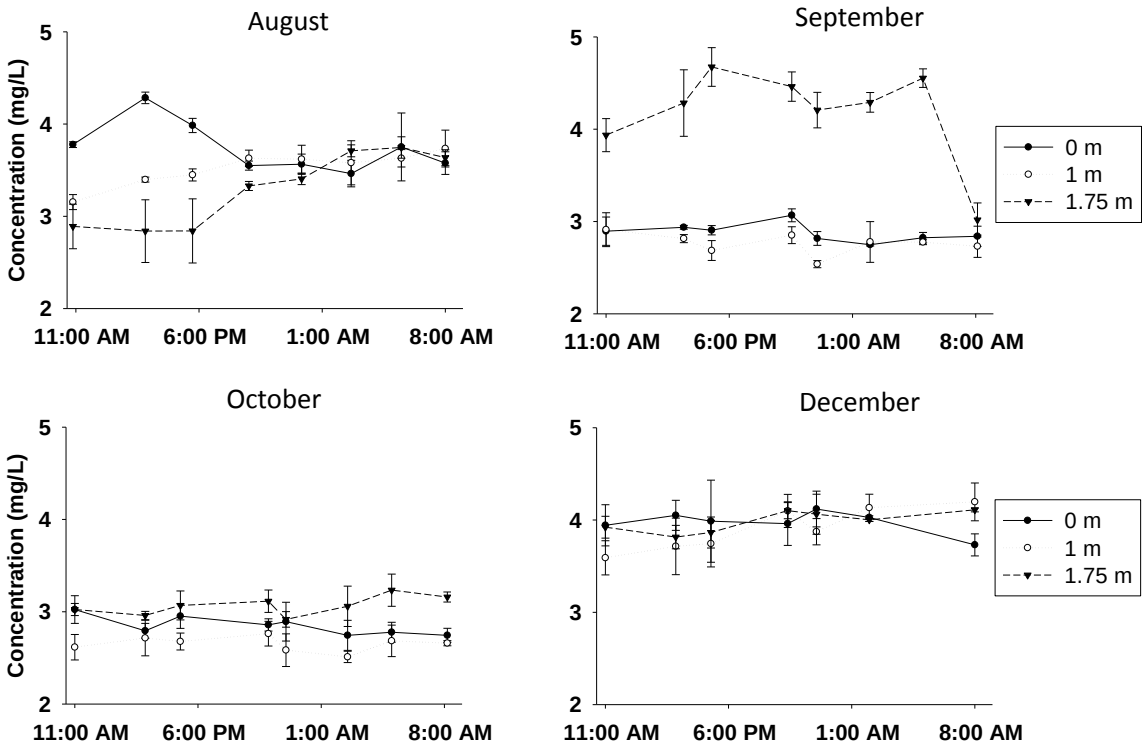

Figure 2.6: Significant diel differences in DOC concentration were observed during August. These were a result of a combination of high primary production near the surface and microbial utilization at the bottom. Sediment production of DOC was higher during September resulting from more carbon being released than was utilized. The sharp decrease at 8:00 am during September was most likely an artifact from the sampling process.

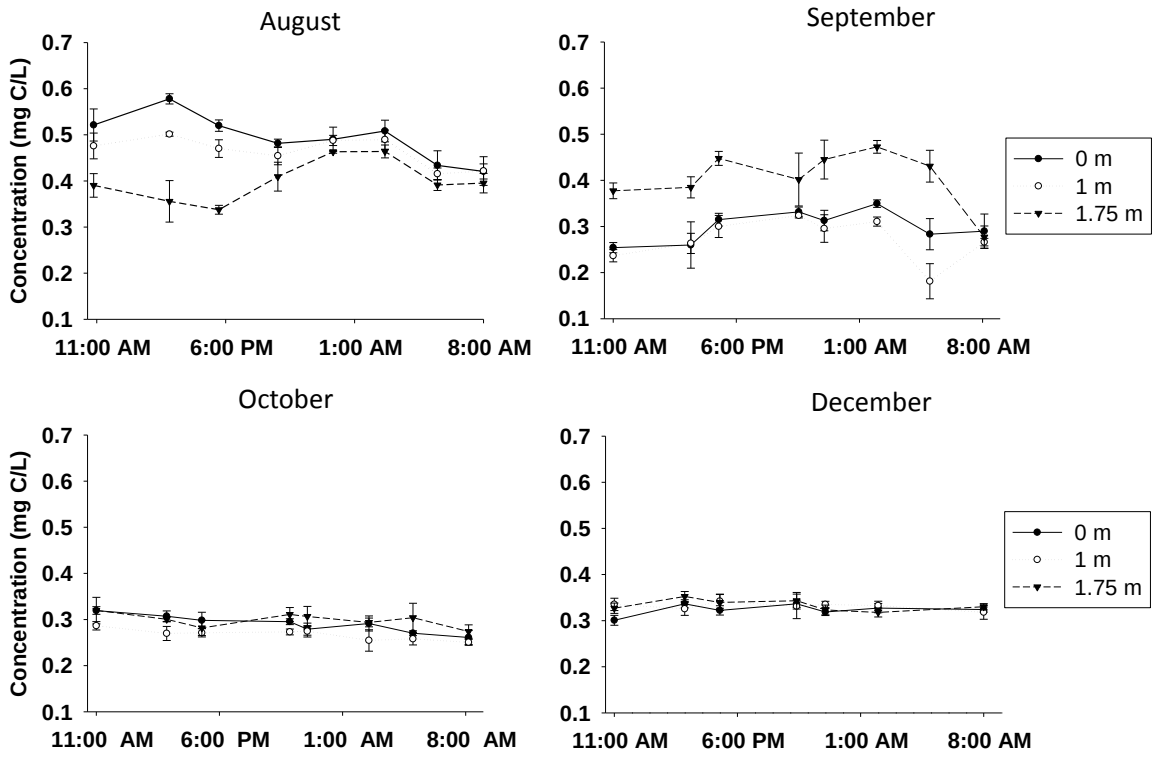

Figure 2.7: Monosaccharide concentrations exhibited diel patterns during August due to extracellular release of carbohydrates. During September, monosaccharide concentrations near the sediment were significantly higher due to carbohydrate release.

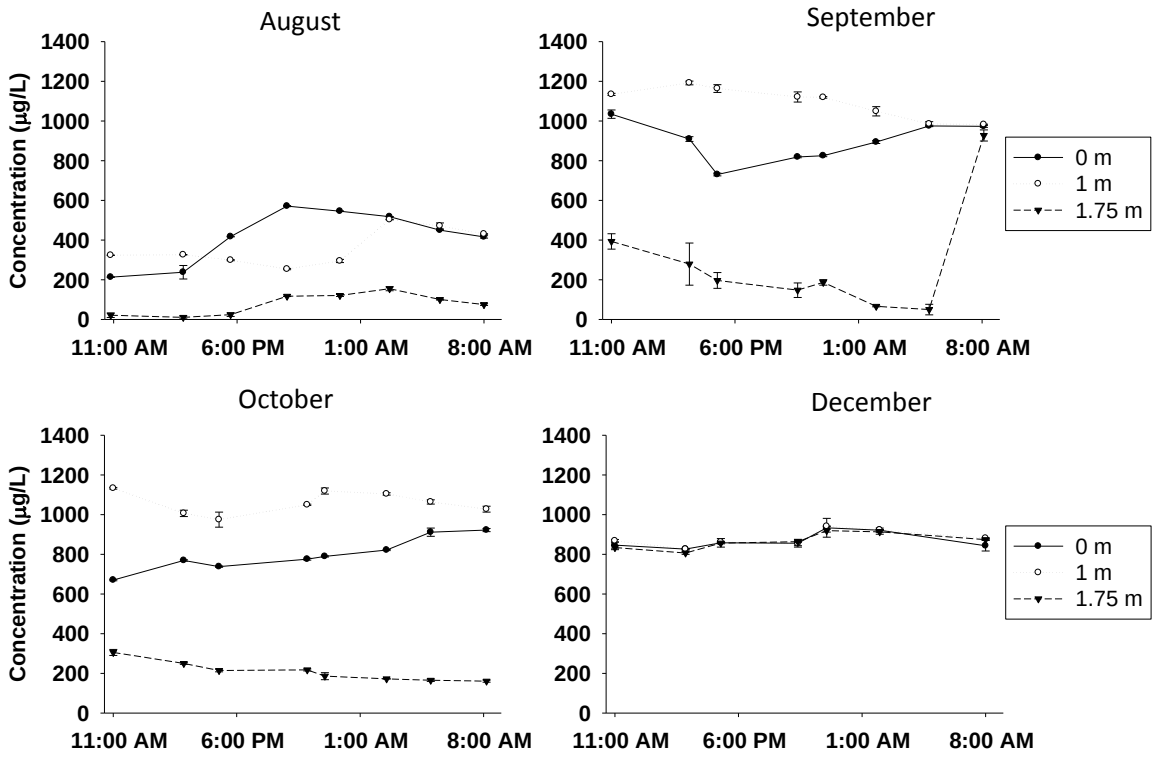

Figure 2.8: NO_3 concentrations remained low near the sediment for all months except December. These low NO_3 concentrations are a good indicator of NO_3 removal, either through assimilation or denitrification, within these SAV beds. The sharp increase at 8:00 am during September was most likely an artifact from the sampling process.

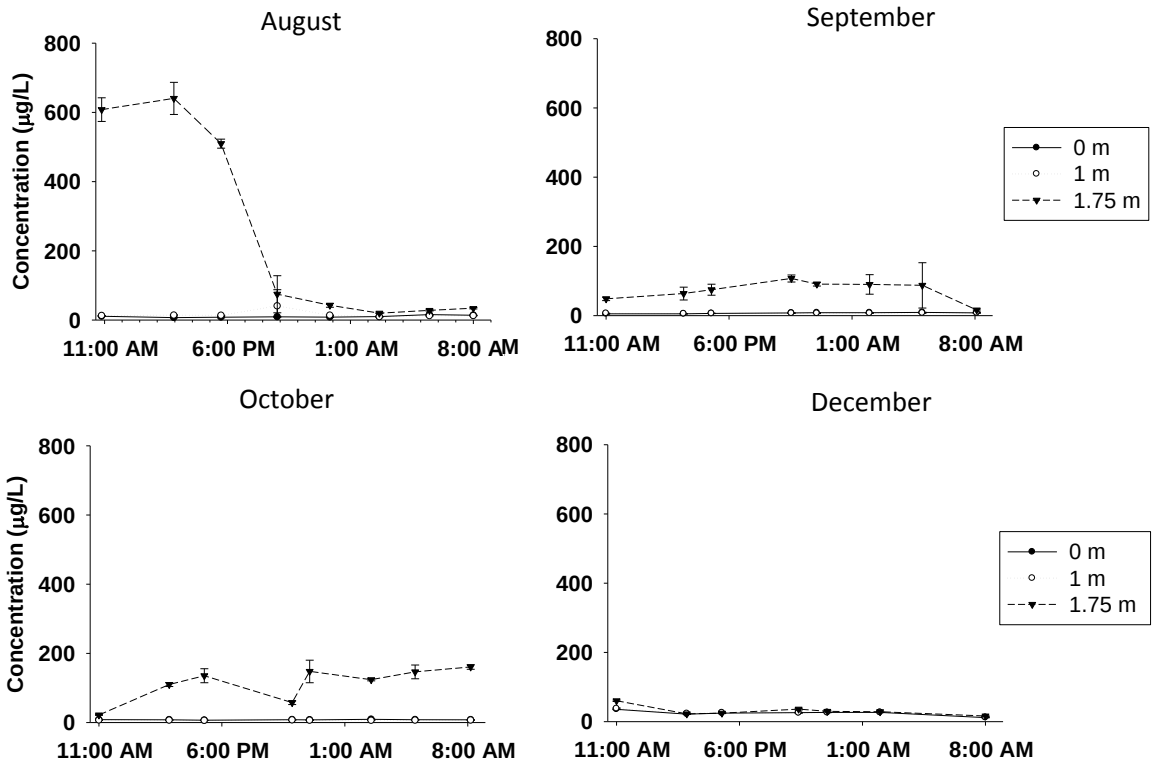

Figure 2.9: NH_4 was significantly higher near the sediment during all months except December. The large increase near the sediment during August, coupled with lower DOC concentrations, indicate that remineralization of DOM is occurring within these SAV beds.

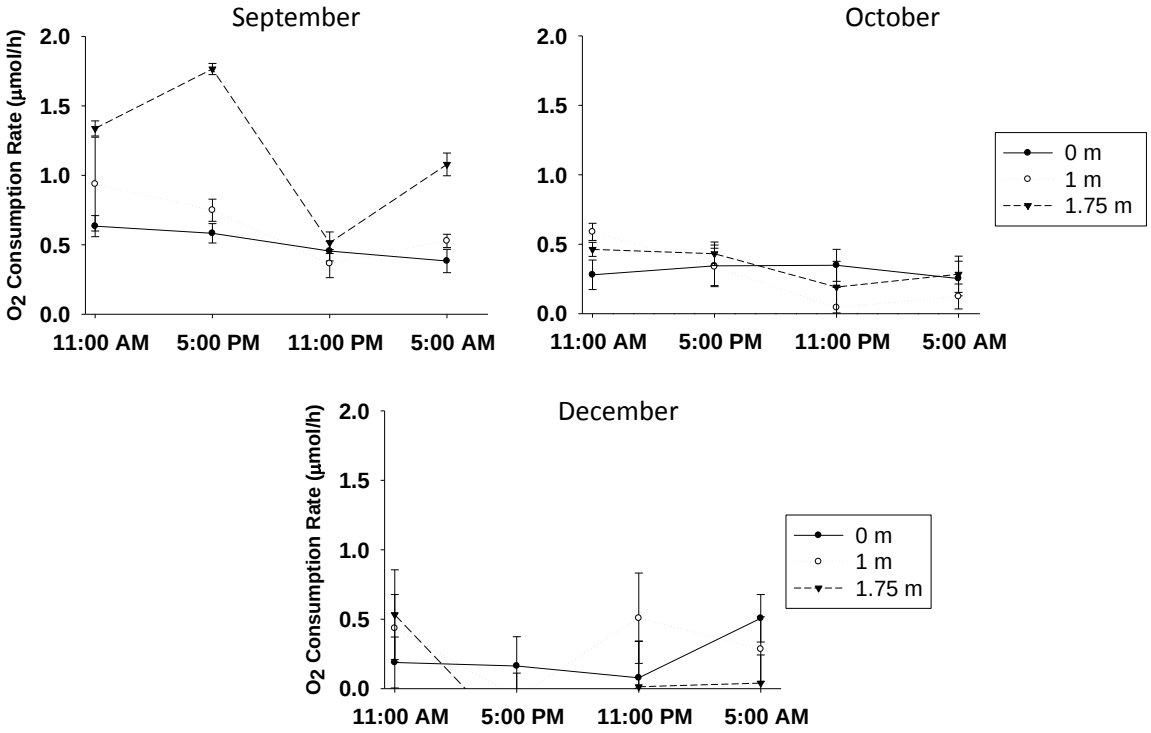

Figure 2.10: O_2 consumption rates were higher near the sediment during September. These higher rates indicate higher levels of microbial metabolism and a more labile carbon source for the microbes.

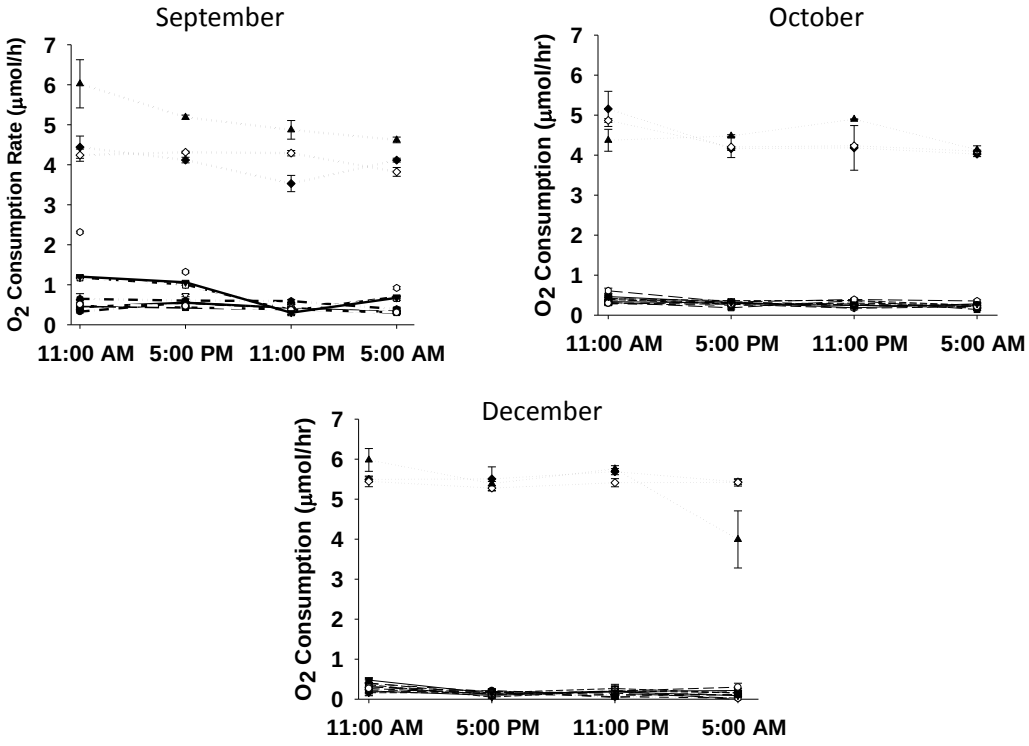

Figure 2.11: Carbon addition greatly stimulated microbial metabolism at all times and depths. The increases in O_2 consumption rates indicate that the microbial communities present within the SAV beds were limited by carbon.

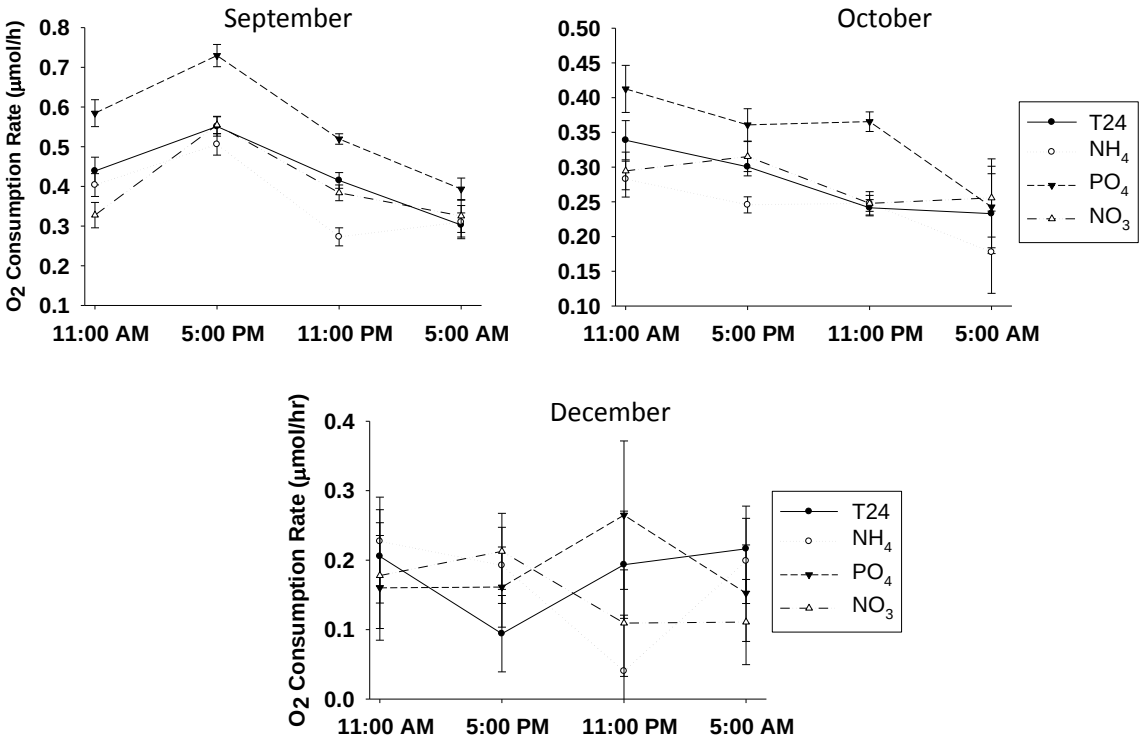

Figure 2.12: P addition stimulated microbial metabolism at most times and depths during September and October. These are examples of this stimulation at 0 m. The increased metabolism suggests that these communities were co-limited by P.

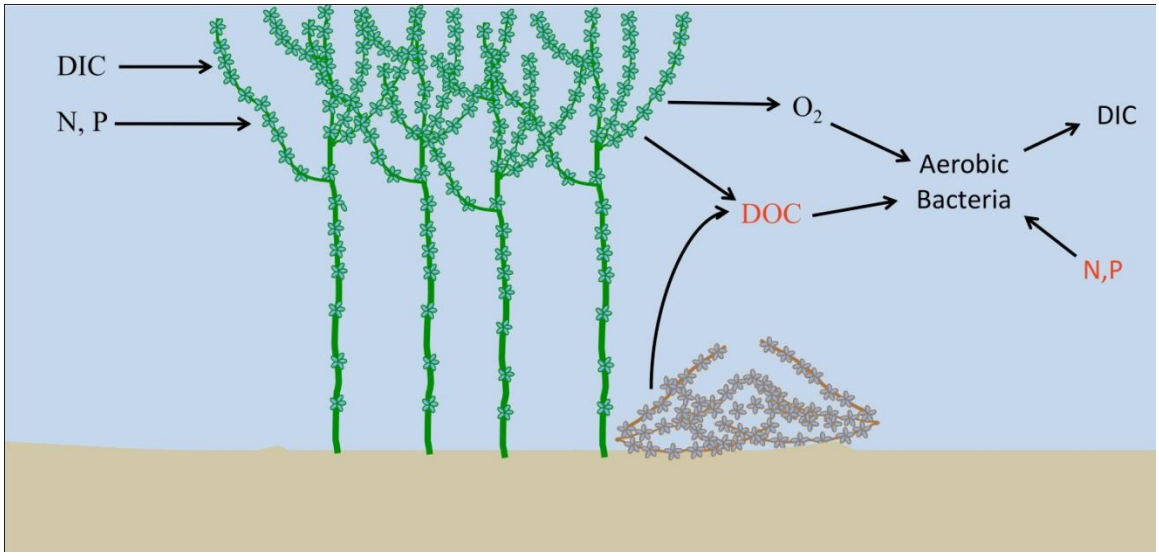

Figure 2.13: A conceptual model showing nutrient and carbon dynamics within a *Hydrilla* bed with an associated aerobic bacterial community. *Hydrilla* utilizes DIC, N, and P from the water column during photosynthesis and also releases O_2 and DOC during photosynthesis. DOC is also released by *Hydrilla* during senescence. The microbial community in the water column utilizes some of the products produced by *Hydrilla* (DOC and O_2) while needing some of the same nutrients as *Hydrilla* (N and P).

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CHAPTER 3

LEACHING AND BIOAVAILABILITY OF DISSOLVED ORGANIC CARBON AMONG FRESHWATER AUTOTROPHIC MACROPHYTES[1]

[1]Shivers, S.D., S.P. Opsahl, A.P. Covich, S. Wilde, and T. Rasmussen. To be submitted to *Aquatic Microbial Ecology*.

Abstract

The concentration and bioavailability of dissolved organic carbon (DOC) that is released after the death of aquatic vegetation can vary greatly among plant types. The response by microbial communities in terms of metabolism varies depending on the bioavailability of DOC. In order to evaluate these differences in microbial metabolism, a series of O_2 consumption experiments were conducted on leachates from four different primary producers (*Hydrilla*, *Typha*, *Potamogeton*, and *Lyngbya*); additionally, DOC and monosaccharide utilization were measured for each experiment. Nutrient concentrations in leachates varied significantly among sample types with *Hydrilla* having the highest NO_3, PO_4, and monosaccharide concentrations and *Typha* the highest DOC concentration. O_2 consumption also varied significantly among producer types with *Hydrilla* having the highest rates and *Potamogeton* the lowest rates. Therefore the origin of the leachates determined how quickly the microbial community utilized the leachate. The findings of this study help to explain the nutrient and carbon dynamics within aquatic ecosystems and some of the effects that different types of autotrophs have on nutrient cycling.

Introduction

Freshwater ecosystems contribute significantly to the global carbon cycle even though they comprise a small proportion of the surface of the Earth (Cole et al. 2007). Because of the coupling of inland waters to terrestrial ecosystems, inland waters serve as integrators on a landscape scale and function as sentinels for a changing climate (Schindler 2009; Williamson et al. 2008). Inland waters store carbon through burial in the sediment and help regulate biogeochemical cycling on a global scale (Tranvik et al. 2009).

One of the principal methods for the regulation of nutrient cycling is the uptake and release of nutrients by aquatic vegetation (Flindt et al. 1999). Aquatic vegetation is an integral part of aquatic ecosystems. Submerged, emergent, and free-floating vegetation affect an array of physical and chemical characteristics in the water in which they grow (Carpenter and Lodge 1986). These effects include changes in light penetration, water flow, and nutrient cycling (Havens 2003; Madsen et al. 2001) and can be strong enough to regulate water clarity on a lake wide scale (Scheffer et al. 1993). One of the ways in which these effects are produced is through the leaching of nutrients from plants, and this leaching occurs while the plant is alive and during senescence (Barko et al. 1986; Godshalk and Wetzel 1978; Mann and Wetzel 1996).

Plant leachates are utilized by bacteria and are crucial for nutrient cycling because they make nutrients, particularly dissolved organic carbon, available to higher trophic levels by incorporating these elements into their biomass, which is consumed by herbivores and omnivores (Benner et al. 1986; Findlay et al. 1986). Microbial utilization of leached material can be fast and yield a high bacterial growth efficiency (Ogawa et al. 2001). Plant leachates can differ greatly in chemical composition and this, in turn, can cause the leachate to function differently (Maie et al. 2006; Opsahl and Benner 1999; Stepanauskas et al. 2000).

The purpose of this study was to determine the concentration of nutrients (DOC, NO_3, NH_4, and PO_4) released through leaching from different plant types (emergent, submerged, and free-floating), and to assess the bioavailability of carbon to microbial communities using a series of experiments focusing on leachate from collected plant material which measured the O_2 consumption of the microbial community. Monosaccharide utilization rates were measured because monosaccharides are a relatively labile form of DOC that can help to explain the differences in microbial metabolism.

Methods

Site Description

Lake Seminole is a 15,216-hectare impoundment located in southwestern Georgia within the Apalachicola-Chattahoochee-Flint (ACF) Basin (Figure 3.1). Lake Seminole is a run-of-the-river reservoir whose primary functions are to aid in navigation and provide hydroelectric power. The mean depth of Lake Seminole is 3 m and the maximum depth is 10.7 m (Sammons et al. 2005; McEntire, 2009). The major surface inflows into Lake Seminole are the Chattahoochee River, Flint River, and Spring Creek. These tributaries drain a combined land area of 46,141 square kilometers. Groundwater also contributes a substantial component of flow into the lake (Torak et al. 2006). Vegetation has covered large areas of the major inflows of Lake Seminole: 46% of the Chattahoochee River, 38% of the Flint River, and 89% of Spring Creek in a 1997 survey by the USACE (Brown and Maceina 2002).

Plant Collection and Leaching

This study involved collecting plant material from four sites within the lake (Figure 3.2). Living biomass was collected during October 2009 from Lake Seminole for the following autotrophs: *Hydrilla verticillata* (hydrilla), *Potamogeton illinoensis* (Illinois pondweed), *Typha*

spp. (cat-tails), and from *Lyngbya* spp., which form cyanobacterial mats. The plants were transported on ice to the Joseph W. Jones Ecological Research Center where they were washed with deionized water, weighed, and placed in a drying oven at 45°C. The samples were dried for at least 48 hrs before dry weights were recorded.

5 g of dried plant material were placed in 1 L glass beakers and 900 mL of ultrapure H_2O were added. The beakers were covered and refrigerated between 0 and 5°C in the dark for 14 days. The plant material was then removed from the beakers and returned to the drying oven to be weighed after drying. The leachate was filtered through a 0.22 μm nitrocellulose filter to remove most bacteria and stored in 1 L Nalgene polycarbonate bottles below 0°C.

Incubations with Leachates

Incubations to assess the bioavailability of carbon from the different leachates were performed using biological oxygen demand (BOD) bottle experiments. Water collected from Lake Seminole was filtered through a pre-ashed 0.7 μm filter to remove particulate matter and retain most of the microbial community. Then 6 mL of the filtered water were added to BOD bottles to serve as a microbial inoculum. Plant leachate was added so that the DOC concentration was approximately 3 mg/L. Based on previous analysis of the different leachates, nutrient amendments were added (0.06 M NH_4Cl, 0.06 M $C_6H_{12}O_6$, 0.06 M $Na_2HPO_4 \cdot 5H_20$, and 0.06 M $NaNO_3$) to maintain equivalent nutrient concentrations among treatments caused by the differences in plant leachate composition. The remainder of the bottle was filled with artificial lake water (Smart and Barko 1985). All bottles were incubated in the dark for 168 hours (7 days) at 27.5°C. Two controls were also included in this experiment. The first was 100% lake water (Lake only); the second was 6 mL lake water, nutrient amendments, and the remainder artificial lake water (Art Lake Nut). DOC and monosaccharide concentrations were measured in

triplicate and O_2 consumption was measured in duplicate. Every 24 hrs, one set of three bottles was removed and refrigerated below 5 °C to stop microbial activity. After 168 hrs, these bottles were filtered through 0.22 μm pre-ashed glass fiber filters to remove microbes. These bottles were stored under appropriate conditions (0-5 °C for DOC and below 0 °C for monosaccharides) until analysis. Additionally, during the experiment, two bottles were removed every 24 hrs and fixed with NaI/NaOH and $MnSO_4$ for Winkler titrations.

Sample and Statistical Analyses – DOC, Monosaccharides, and Bioassays

DOC was measured using a Shimadzu TOC-V total carbon analyzer. Dissolved monosaccharides were measured using the 2,4,6-Tripyridyl-s-triazine (TPTZ) colorimetric method of Myklestad, modified by Hung (Hung and Santschi 2001). Final measurements of absorbance were made using a Shimadzu UV-2101 spectrophotometer. Dissolved oxygen concentrations were determined through titration utilizing the Winkler method and a Mettler Toledo DL50 titrator (Pomeroy et al. 1994). Graphic visualization of data was constructed using SigmaPlot (SigmaPlot ver. 11). Analysis of variance (ANOVA) with a post hoc Tukey's HSD test was executed on the data using the statistical packages of R (R ver. 2.12.0).

Results

Nutrient Concentrations in Leachates

Nutrient concentrations varied significantly among the different plant types (Table 3.1). The *Typha* leachate contained the highest concentration of DOC (642.5 mg/L) and the lowest concentrations of NH_4 (0.128 mg/L) and NO_3 (0.0329 mg/L). *Hydrilla* had the lowest concentration of DOC (171.9 mg/L) and the highest concentrations of PO_4 (14.8 mg/L) and NO_3 (0.290 mg/L). *Lyngbya* leachate had the highest NH_4 concentration (18.3 mg/L) and the lowest

PO_4 concentration (0.319 mg/L). *Potamogeton* leachate was intermediate for all nutrient concentrations.

DOC Concentrations

Overall, the DOC concentrations varied significantly over time among the different treatments (ANOVA, $p<0.01$). The DOC concentration of BOD bottles containing *Hydrilla* leachate dropped sharply during the first 24 hrs (from 3.03 mg/L to 1.44 mg/L) and continued to decrease during the next 24 hrs (from 1.44 mg/L to 0.75 mg/L) (Figure 3.3). After 48 hrs, DOC concentrations remained stable for the remainder of the *Hydrilla* incubation (mean = 0.76 mg/L). Bottles containing *Lyngbya* leachate also exhibited sharply decreasing DOC concentrations during the first 24 hrs (from 2.56 mg/L to 0.70 mg/L), but after 24 hrs DOC decreased at a slower rate for the next 120 hrs (mean decrease per 24 hours = 0.6 mg/L). DOC concentrations for *Potamogeton* bottles decreased less sharply than *Hydrilla* or *Lyngbya* for the first 24 hrs (from 2.77 mg/L to 1.88 mg/L), but they continued to decrease steadily over the next 96 hrs (mean decrease of 0.15 mg/L per 24 hrs) before leveling off after 120 hrs. DOC concentrations of *Typha* decreased slower but longer than the other plant types; these concentrations decreased an average of 0.63 mg/L per 24 hrs for the first 72 hrs, 0.13 mg/L for the next 48 hrs, and remained stable for the final 48 hrs. For the lake water samples, DOC decreased slightly over the duration of the experiment (from 2.59 mg/L to 2.29 mg/L). The samples composed of artificial lake water and 10% lake water decreased by 0.04 mg/L over the first 24 hrs, and DOC concentrations were ~ 0 mg/L for the remainder of the experiment.

Monosaccharide Concentrations

Monosaccharide concentrations varied significantly over time based upon treatment (ANOVA, $p<0.01$). The initial monosaccharide concentrations of the *Hydrilla* and *Lyngbya*

leachates were similar (0.82 mg C/L and 0.71 mg C/L, respectively), decreased at the same rate over the first 24 hrs (0.52 mg C/L), and both remained relatively stable after 48 hrs (Figure 3.4). Monosaccharide concentrations did have a slightly larger decrease between 24 and 48 hrs for *Hydrilla* (0.09 mg C/L) as compared to *Lyngbya* (0.04 mg C/L). Monosaccharide concentrations of *Potamogeton* leachates were initially higher than *Hydrilla* or *Lyngbya* (1.45 mg C/L). But, concentrations decreased similarly over the first 24 hrs (0.55 mg C/L), were slightly higher over the next 24 hrs (0.14 mg C/L) than *Hydrilla* or *Lyngbya*, and were stable for the remainder of the experiment. Initial *Typha* leachate monosaccharide concentrations were higher than the other plant types (1.82 mg C/L). Concentrations decreased at a slow rate over the first 24 hrs (0.07 mg C/L), decreased faster over the next 48 hrs (0.58 mg C/L and 0.49 mg C/L), and then decreased slower over 48 hrs before becoming stable for the final 48 hrs. Neither control group experienced significant concentration changes over the course of the experiment, but the 100% lake water samples were significantly higher (0.48 mg C/L) than the predominantly artificial lake water samples (0.06 mg C/L).

O_2 Consumption

O_2 consumption rates were highest overall for bottles containing *Hydrilla* leachate. These bottles lost 84.91 μM of O_2 during the first 72 hrs and 189.57 μM of O_2 over 168 hrs (Figure 3.5). *Lyngbya* had a higher rate for the first 72 hrs (117.78 μM of O_2 lost), but slowed considerably afterwards (152.66 μM total O_2 decrease). Conversely, bottles containing *Typha* leachate decreased at a slower rate over the first 72 hrs (70.19 μM O_2), but decreased at a more steady rate overall (127.62 μM O_2). *Potamogeton* leachate saw the slowest O_2 consumption in the first 72 hrs (68.57 μM O_2) and the slowest overall (106.76 μM O_2). The O_2 consumption rates of both controls were low, but steady for the duration of the experiment.

Discussion

Organic Carbon Utilization and Bioavailability

All leachates stimulated O_2 consumption compared to the controls, which means that bioavailable carbon was present in all leachates. However, the patterns of DOC and monosaccharide utilization varied significantly among plant types, and this can indicate differences in bioavailability to microbial communities. *Lyngbya* incubations utilized DOC and monosaccharides the fastest over the first 24 hrs but then slowed greatly for the duration of the experiment. These carbon utilization rates imply that there was labile carbon present initially and quickly taken up by microbes. This is supported by the fastest O_2 consumption rates during the first 24 hrs, followed by decreased O_2 consumption during the next 24 hrs, and low rates after the first 48 hours. Although other studies have examined the effects of nutrients on the growth and nitrogen fixation rates of *Lyngbya* and effects of *Lyngbya* on other organisms (Camacho and Thacker 2006; Cowell and Botts 1994; Elmetri and Bell 2004); few, if any, studies have investigated the quantity and quality of nutrients leached from *Lyngbya*. Considering the rapid use of carbon from *Lyngbya* and that cyanobacterial blooms could possibly be more frequent as a result of climate change, further studies to examine the effects of *Lyngbya* on nutrient cycling should be considered (Paerl and Huisman 2009).

Incubations containing *Hydrilla* leachate also utilized DOC and monosaccharides extremely quickly over the first 24 hrs, but continued at a rapid pace during the next 24 hrs. The monosaccharide:DOC ratio of 0.27 is similar to *Lyngbya*, which also had similar initial carbon utilization rates. However, O_2 consumption rates were the highest overall for *Hydrilla* leachate incubations. The fast initial rates of O_2 consumption remained high over the next 24-hr period, and were higher than the other plant types for the remainder of the experiment. These rates also

support that *Hydrilla* leachates contained more bioavailable C than the other plant types. Other studies have assessed the effects that *Hydrilla* can have on nutrient concentration and availability, but few have directly assessed the bioavailability of carbon to the microbial community (Barko et al. 1988; Gu 2006; Takamura et al. 2003).

Typha incubations had the most gradual utilization of DOC and monosaccharides of all the different samples. After a brief lag period during the first 24 hrs, monosaccharide utilization was higher for the next 48 hrs before decreasing slowly for the remainder of the experiment. This pattern implies that there was less bioavailable carbon for immediate uptake, but semi-labile carbon did become available over time. This change in carbon quality is supported by the very slow O_2 consumption rate during the first 24 hrs and the higher rates over the next 72 hrs. These results are consistent with a study by Mann and Wetzel (1996) that showed lower bacterial growth efficiencies that implied lower lability of *Typha* DOC. A potential explanation for these data is the physical structure of the *Typha* plant. Because *Typha* is an emergent plant, more structural components are necessary to maintain plant rigidity. These components are more recalcitrant than other forms of carbon and are not immediately available for uptake. This is supported by other studies that have shown *Typha* to decompose slowly compared to other aquatic macrophytes (Alvarez and Becares 2006; Chimney and Pietro 2006).

Potamogeton incubations had the lowest rates of carbon utilization. The first 24 hrs were the slowest of all samples for DOC utilization, although monosaccharide usage was comparable to the other samples. Both DOC and monosaccharide utilization rates were low after the first 24 hours. These rates imply that there was a small quantity of immediately bioavailable carbon, and very little became available afterwards. This is supported by the lowest overall O_2 consumption rates. Like *Typha*, the structural nature of this plant could be responsible for these rates.

Potamogeton is characterized by long stems that have fewer leaves than other submerged vegetation. It is likely that there are more structural components, such as aromatic rings, present in these leachates than the others. The stained, tea-like appearance of this leachate supports this idea.

Nutrient Concentrations of Leachates

Differing plant types can have very different biochemical compositions, which can affect what is leached upon senescence; these differences, in turn, can affect local nutrient cycling (Hooper and Vitousek 1998; Maie et al. 2006). Qualitatively, the leachates in this experiment were quite different and ranged in color from clear with a bluish tint (*Lyngbya*) to dark tea-colored (*Potamogeton*). This coloration implies that these leachates are optically different and that, as a result, they could have different properties (Boyd and Osburn 2004; Coble 2007; Kowalczuk et al. 2003). Quantitatively, the nutrient compositions of the leachates were also different. These differences could be expected considering the different physiologies of these plants. The different nutrient compositions and when those nutrients are released can have an impact on nutrient cycling in the surrounding waters (Carpenter 1980; Carpenter and Lodge 1986). Because *Hydrilla* can cover large spatial areas and have the potential to leach high concentrations of NO_3 (.290 mg/L), PO_4 (14.8 mg/L), and NH_4 (13 mg/L), this influx of nutrients upon senescence could affect nutrient cycling on large scales. Nutrients can also be moved throughout the water body by non-rooted species, such as *Lyngbya*. *Lyngbya* releases high concentrations of NH_4 (18.3 mg/L) upon senescence and could be responsible for nitrogen transport throughout a water body. Additionally, *Lyngbya* can fix nitrogen from the air under anaerobic conditions in the benthos (Philips et al. 1992). Therefore, the nutrient composition of primary producers can not only affect shallow areas where macrophytes are more abundant, but

also cycling throughout the lake. Additionally, if spatial coverage and density are sufficiently high, nutrient cycling can be affected on the basin scale.

The availability of labile carbon is important because it serves as an energy source for the microbial community. This study has shown that different plant types release a different quantity and quality of carbon and nutrients upon senescence. Plants that can cover large areas and are densely populated, such as *Hydrilla*, can have significant impacts on large areas of a water body. A more labile carbon source can increase microbial metabolism, which can have direct impacts on nutrient cycling within aquatic systems. One potential impact could be on denitrification rates. A more labile carbon source could easily increase nitrogen removal rates.

The chemical composition of the leachates also had significant effects on their functions. For example, a higher monosaccharide:DOC ratio resulted in a higher microbial metabolism due to the presence of labile carbon. However, the results did not explain the nuances among carbon utilization rates. For example, this study found that the O_2 consumption of *Hydrilla* leachate continued even though DOC and monosaccharide utilization did not. Further research to explore the relationship between the monosaccharide:DOC ratio and O_2 consumption by bacteria would help to explain these differences. Future research to specifically determine the chemical composition (i.e. neutral sugars, total carbohydrates, and proteins) of different plant leachates would also increase the ability to predict their in situ function.

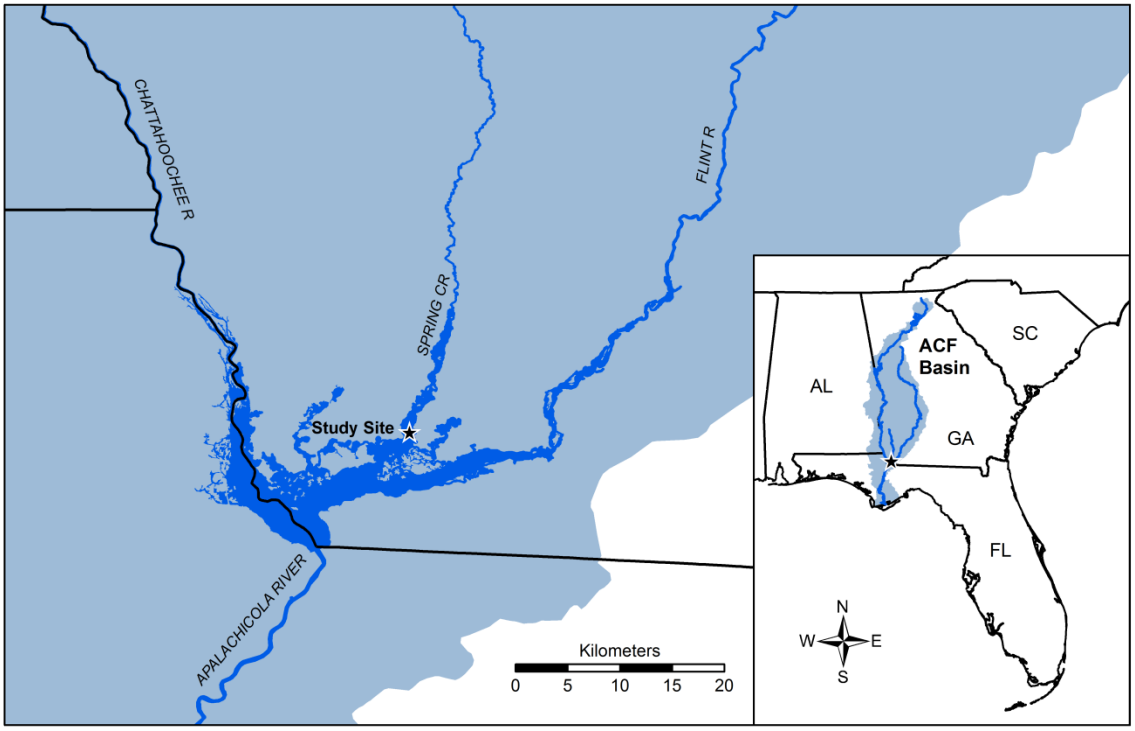

Figure 3.1: Lake Seminole is located in the southwestern corner of Georgia. The main inflows are the Chattahoochee River, Flint River, and Spring Creek, and the main outflow is the Apalachicola River.

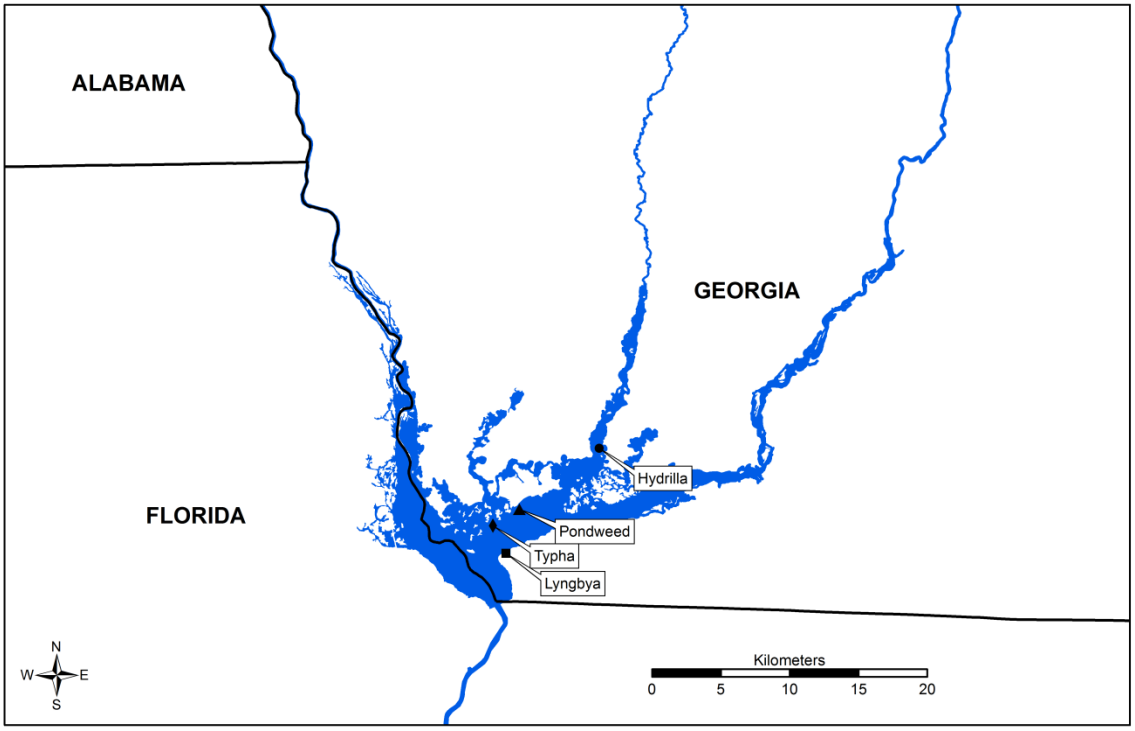

Figure 3.2: Living biomass was collected for four different plant types (*Hydrilla*, *Potamogeton*, *Typha*, and *Lyngbya*) from these locations on Lake Seminole.

	DOC (mg/L)	NH_4 (mg/L)	PO_4 (mg/L)	NO_3 (mg/L)
Potamogeton	319.8	3.09	5.29	0.144
Hydrilla	171.9	13	14.8	0.290
Typha	642.5	0.128	6.83	0.032
Lyngbya	435.8	18.3	0.319	0.035

Table 3.1: Nutrient and DOC concentrations varied significantly between the different plant leachates.

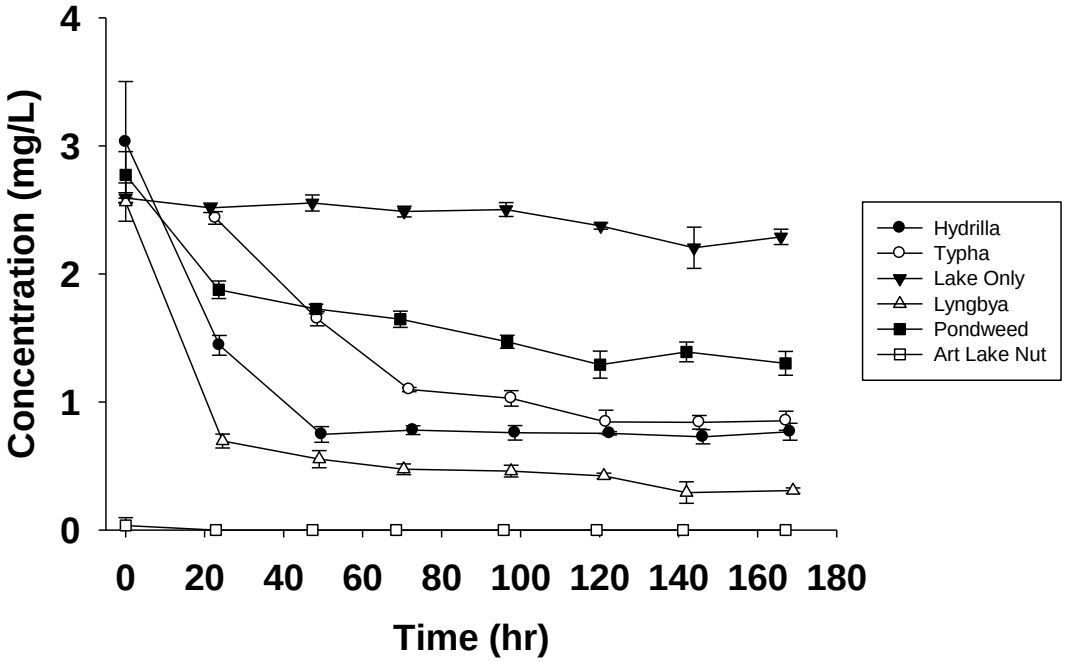

Figure 3.3: DOC concentrations varied significantly between plant types. *Hydrilla* and *Lyngbya* had the fastest and highest overall utilization of DOC.

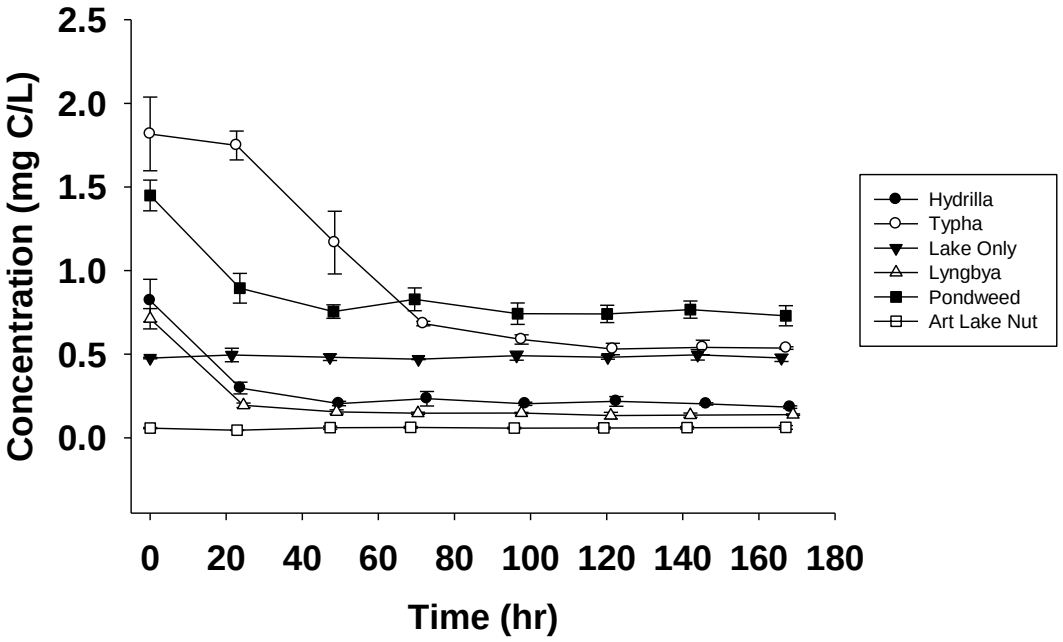

Figure 3.4: The concentration of monosaccharides varied significantly between plant types. *Hydrilla* and *Lyngbya* exhibited fast initial monosaccharide utilization, while *Typha* was slower and more sustained.

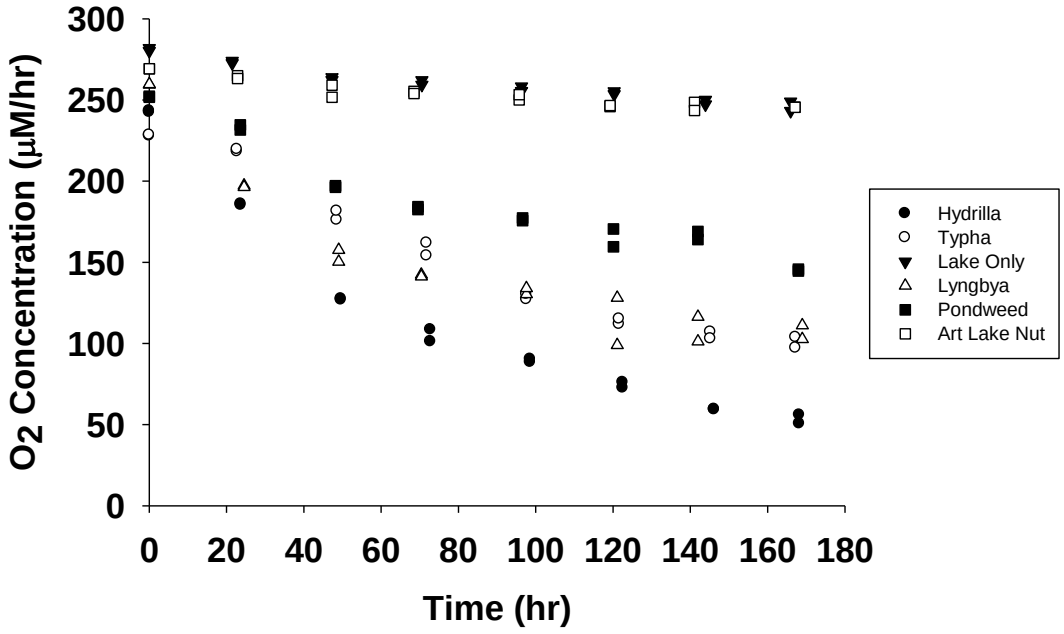

Figure 3.5: O_2 consumption was significantly different between different plants. *Hydrilla* leachate had the highest overall O_2 consumption rates, followed by *Typha* and *Lyngbya*, and *Potamogeton* had the lowest.

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CHAPTER 4

CONCLUSIONS AND SUMMARY

Inland waters have been shown to make significant contributions to the global carbon cycle and to biogeochemical cycling in general (Cole et al. 2007; Tranvik et al. 2009). Due to extensive spatial coverage and high productivity, submerged aquatic vegetation can also contribute to and affect biogeochemical cycling (Carpenter and Lodge 1986; Takamura et al. 2003). This study sought to assess the effects of submerged aquatic vegetation (SAV) on nutrient cycling within Lake Seminole. More specifically, the effects of *Hydrilla verticillata* on the physical and chemical characteristics of the water column were evaluated on diel and seasonal time scales. Additionally, the bioavailability of carbon from several plant types to the microbial community was assessed.

This study utilized hydrilla beds in order to assess the effects of SAV on water column dynamics. The effects of SAV on the physical parameters of the water column were large and seasonally distinct. During times of high primary productivity, such as August and September, large differences were observed between the surface and bottom of the water column. DO and pH were higher at the surface and lower near the sediment. High levels of primary production contributed to the increases in DO and pH at the surface, and decomposition of organic material removed DO at the bottom. This variation became less pronounced later in the season, and differences between depths were not significantly different in December.

The effects of SAV on the chemical parameters of the water column were also significantly different during August and September. Large diel changes in DOC and

monosaccharide concentrations were observed during August. Higher concentrations were present during the day at the surface due to carbon release during photosynthesis, and concentrations were lower near the sediment as carbon was consumed faster than it was produced. During September, DOC and monosaccharide concentrations were much higher near the sediment than the other depths. These lower concentrations were likely due to decomposition releasing carbon into the water (Bertilsson and Jones Jr. 2003). These differences are important because they provide a labile source of energy for the microbial community. There were no large changes in DOC or monosaccharide concentrations during October or December. NO_3 concentrations were significantly lower near the sediment during August, September, and October, but not December. These reduced NO_3 concentrations are important because denitrification can remove NO_3 from an ecosystem thereby mitigating high NO_3 concentrations that can occur in aquatic ecosystems (Kufel and Kufel 2002; Seitzinger 1988). Carbon bioavailability was also found to have diel and seasonal patterns. Bioavailability was found to be higher during the day and near the sediment during September. These patterns correlate with higher photosynthetic activity at the surface and the large benthic releases of carbon that were observed during September. Increased bioavailability of carbon to microbial communities can result in a higher microbial community metabolism. This increased metabolism can result in changes in the biogeochemical cycles, such as denitrification, that can alter this aquatic community (Paerl and Pinckney 1996).

Another purpose of this study was to assess the concentrations of nutrients and carbon and also the bioavailability of carbon from a variety of plant leachates, which included *Hydrilla*, *Typha*, *Lyngbya*, and *Potamogeton*. The plant leachates were found to have extraordinarily different nutrient concentrations. This variability can result in fluctuating local patterns of

nutrient cycling depending upon which plants are present. The carbon bioavailabilities of the leachates were also significantly different. *Hydrilla* leachate was found to have the highest O_2 consumption and thus the greatest bioavailability. This increased bioavailability was further supported by fast utilization of DOC and monosaccharides over a longer period. *Lyngbya* and *Typha* leachates were found to have similar bioavailability based on O_2 consumption. This result is interesting because DOC and monosaccharide utilization, as well as life histories, are quite dissimilar between the two. However, the dynamics of carbon utilization show that *Lyngbya* and *Typha* make different chemical contributions to the DOM pool, even though they are similar in overall bioavailability. *Potamogeton* was found to have the lowest bioavailability. This lower bioavailability was supported by the lowest carbon utilization rates of all the plant types. This result is most likely due to the physical structure of the plant. It is clear from this experiment that different plants contribute differently to the DOM pool. Also, the corresponding microbial metabolism will be different depending upon which plant is present in the ecosystem.

Overall, this study supports the idea that inland waters make significant contributions to the global carbon cycle, and that SAV beds are extraordinarily dynamic over both diel and seasonal timescales. These dynamics result in different nutrient and carbon concentrations and bioavailabilities. These differences can result in variable microbial metabolisms over an annual cycle, which means that nutrient cycling will also be different during the year. This study also found that different plant leachates can have different carbon bioavailabilities. Therefore, the biogeochemical cycling, occurring within inland waters, can be affected at multiple levels by submerged aquatic vegetation. Future research could include direct assessment of denitrification within SAV beds. That research could then be used to approximate denitrification on a reservoir scale. Because many of these cycles have direct effects upon the food web, a thorough study of

food web dynamics within SAV beds could help to further explain the impact that SAV can have on aquatic ecosystems.

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Appendix A

		August		September		October		December	
		Temp (°C)	DO (mg/L)	Temp (°C)	DO (mg/L)	Temp (°C)	DO (mg/L)	Temp (°C)	DO (mg/L)
	0 m	29.34	12.03	28.66	12.72	21.45	8.8	15.25	9.59
11 am	1 m	28.43	8.21	27.77	9.28	20.91	9.78	15.24	9.6
	1.7 m	26.33	0.65	26.82	2.25	20	0.85	15.23	9.16
	0 m	33.04	16.81	30.09	15.59	23.18	9.9	15.4	10.53
2 pm	1 m	29.15	12.21	27.95	11.54	21.32	11.45	15.41	10.35
	1.7 m	26.26	0.16	26.24	0.68	20.02	0.86	15.43	9.48
	0 m	33.06	15.28	30.05	15.62	23.11	10.4	15.09	10.51
5 pm	1 m	29.28	11.89	28.04	11.79	21.42	13.85	15.09	10.44
	1.7 m	26.25	0.53	26.49	0.46	20.12	1.68	15.09	10.45
	0 m	31.29	15.19	29.21	13.98	22.55	10.61	14.76	10.62
8 pm	1 m	29.99	14.9	27.98	11.57	21.36	12.86	14.77	11.06
	1.7 m	27.01	1.72	26.47	0.75	20.12	1.9	14.8	10.28
	0 m	30.36	11.89	28.49	12.91	22.18	10.51	14.19	9.82
11 pm	1 m	29.83	13.7	27.94	10.82	21.38	11.29	14.23	9.97
	1.7 m	27.05	3.4	26.4	1.11	20.16	2.03	14.27	9.7
	0 m	29.84	11.72	28.27	12.23	22	10.35	13.97	9.65
2 am	1 m	29.81	11.43	28.02	10.92	21.36	11.41	13.99	9.66
	1.7 m	27.83	5.01	26.62	1.06	20.2	2.26	14.05	9.32
	0 m	29.38	11.14	27.85	10.75	21.67	10.59	NA	NA
5 am	1 m	29.37	11.08	27.83	10.11	21.34	10.74	NA	NA
	1.7 m	26.65	1.13	26.1	0.35	20.25	1.53	NA	NA
	0 m	29.12	9.93	27.66	9.83	21.6	10.62	12.97	9.52
8 am	1 m	29.11	9.96	26.82	10.21	21.36	10.54	13	9.45
	1.7 m	27.99	5.89	26.21	0.55	20.23	1.74	13.01	9.41

Table A.1: Temperature and dissolved oxygen (DO) measured between August-December at the surface, intermediate, and above sediment depths for all time points.

		August		September		October		December	
		Cond (ms/cm)	pH	Cond (ms/cm)	pH	Cond (ms/cm)	pH	Cond (ms/cm)	pH
	0 m	0.101	9.1	0.138	8.5	0.156	8.02	0.203	8.17
11 am	1 m	0.109	8.07	0.16	7.79	0.168	7.95	0.203	8.16
	1.7 m	0.244	7.04	0.167	7.36	0.2	7.72	0.209	8.14
	0 m	0.114	9.52	0.126	9.07	0.149	8.44	0.203	8.28
2 pm	1 m	0.104	8.89	0.169	7.89	0.157	8.59	0.204	8.24
	1.7 m	0.228	7.2	0.193	7.49	0.206	7.68	0.205	8.17
	0 m	0.114	9.83	0.117	9.28	0.148	8.52	0.204	8.34
5 pm	1 m	0.102	9.2	0.158	8.16	0.15	8.61	0.204	8.34
	1.7 m	0.234	7.18	0.193	7.86	0.198	7.89	0.204	8.33
	0 m	0.103	9.51	0.118	9.08	0.148	8.48	0.204	8.3
8 pm	1 m	0.099	9.54	0.158	8.09	0.15	8.38	0.204	8.28
	1.7 m	0.179	7.84	0.193	7.64	0.2	7.61	0.205	8.19
	0 m	0.1	9.21	0.121	8.87	0.149	8.42	0.207	8.16
11 pm	1 m	0.097	9.3	0.157	8.01	0.162	8.28	0.207	8.17
	1.7 m	0.186	7.42	0.197	7.82	0.195	7.7	0.207	8.16
	0 m	0.101	9.12	0.126	8.71	0.15	8.37	0.207	8.09
2 am	1 m	0.099	9.1	0.151	8.09	0.16	8.27	0.207	8.08
	1.7 m	0.126	7.67	0.171	7.8	0.193	7.64	0.209	8.07
	0 m	0.099	9.01	0.138	8.29	0.154	8.29	NA	NA
5 am	1 m	0.098	8.97	0.145	8.07	0.164	8.09	NA	NA
	1.7 m	0.206	7.19	0.21	7.5	0.2	7.64	NA	NA
	0 m	0.1	8.85	0.145	8.01	0.155	8.27	0.206	8.04
8 am	1 m	0.1	8.84	0.146	7.86	0.16	8.03	0.206	8.05
	1.7 m	0.107	8.15	0.205	7.4	0.2	7.67	0.206	8.07

Table A.2: Conductivity and pH were measured at the surface, intermediate, and above sediment depths for all time points during the months of August-December.

		August		September		October		December	
		NO_3 (µg/L)	NH_4 (µg/L)	NO_3 (µg/L)	NH_4 (µg/L)	NO_3 (µg/L)	NH_4 (µg/L)	NO_3 (µg/L)	NH_4 (µg/L)
	0 m	213	10.8	1034.4	5.1	670.2	8.4	846.4	35.5
11 am	1 m	323.3	12.7	1134	6.6	1132.9	6.9	867.9	37.5
	1.7 m	21.2	608	393	48.3	305.5	21.9	834	60.2
	0 m	237.7	7	909	5.1	769	7.9	826.3	21.5
2 pm	1 m	325.7	13.9	1191.7	5.4	1006.2	6.6	826	23.4
	1.7 m	10.2	640.3	278.9	63.7	249.8	109.8	806.2	22.3
	0 m	417.3	8.2	730.3	6.3	737.7	6.7	857.9	24.5
5 pm	1 m	298.7	13.4	1163.3	6.1	974.5	5.8	861.9	25.8
	1.7 m	23.7	509.7	196.3	74.7	214.3	135.3	856.2	24.3
	0 m	571	9.2	818.7	7.7	775.6	8.2	855.8	25.9
8 pm	1 m	253.7	40.5	1121	6.8	1049.3	7.1	853.4	25.3
	1.7 m	116.7	74.6	147.3	107.3	217.2	57.4	863.4	35.9
	0 m	545.3	8.3	824.8	8.3	789.3	7.7	933.6	26.6
11 pm	1 m	294.7	13.6	1119	6.4	1118.9	6.3	940.9	25.9
	1.7 m	120.7	42.6	186.5	90.9	185.8	147.7	919.1	29.7
	0 m	517.7	9.9	894	8.3	821.3	9.2	921.4	26.7
2 am	1 m	503	9.3	1048.7	6.8	1104.2	6	922.6	26.6
	1.7 m	154.7	19.9	65.7	90	172.1	123.7	912.8	28.3
	0 m	450	15.7	975.1	9.2	911.6	8.2	NA	NA
5 am	1 m	471	12.1	984.9	7.5	1063.7	6.5	NA	NA
	1.7 m	100.3	27.7	49.6	87.4	165.1	146.4	NA	NA
	0 m	416	13.8	972.8	8	922.1	7.9	843.1	11.6
8 am	1 m	430.7	12.4	982.3	7.8	1027.4	6.6	880.9	12.5
	1.7 m	74.8	34.2	927	16	160.4	160.6	873.7	16

Table A.3: Mean NO_3 and NH_4 concentrations measured at the surface, intermediate, and above sediment depths at all time points between August-December.

		August		September		October		December	
		DOC (mg/L)	MCHO (mg/L)	DOC (mg/L)	MCHO (mg/L)	DOC (mg/L)	MCHO (mg/L)	DOC (mg/L)	MCHO (mg/L)
	0 m	3.78	0.52	2.9	0.25	3.02	0.32	3.94	0.3
11 am	1 m	3.15	0.48	2.91	0.24	2.62	0.29	3.59	0.34
	1.7 m	2.89	0.39	3.94	0.38	3.02	0.32	3.92	0.33
	0 m	4.28	0.58	2.94	0.26	2.79	0.31	4.05	0.34
2 pm	1 m	3.4	0.5	2.81	0.26	2.72	0.27	3.71	0.33
	1.7 m	2.84	0.36	4.28	0.45	2.96	0.3	3.82	0.35
	0 m	3.98	0.52	2.91	0.31	2.95	0.3	3.99	0.32
5 pm	1 m	3.45	0.47	2.69	0.3	2.68	0.27	3.74	0.34
	1.7 m	2.84	0.34	4.67	0.4	3.07	0.28	3.87	0.34
	0 m	3.55	0.48	3.07	0.33	2.86	0.3	3.96	0.34
8 pm	1 m	3.63	0.45	2.85	0.32	2.76	0.27	4.1	0.33
	1.7 m	3.33	0.41	4.46	0.45	3.11	0.31	4.1	0.34
	0 m	3.56	0.49	2.82	0.31	2.89	0.28	4.11	0.32
11 pm	1 m	3.62	0.49	2.54	0.3	2.58	0.27	3.87	0.34
	1.7 m	3.41	0.46	4.21	0.45	2.92	0.31	4.06	0.32
	0 m	3.46	0.51	2.75	0.35	2.74	0.29	4.03	0.33
2 am	1 m	3.58	0.49	2.78	0.31	2.51	0.25	4.13	0.33
	1.7 m	3.71	0.46	4.29	0.47	3.06	0.29	4	0.32
	0 m	3.75	0.43	2.82	0.28	2.78	0.27	NA	NA
5 am	1 m	3.63	0.42	2.78	0.18	2.68	0.26	NA	NA
	1.7 m	3.75	0.39	4.55	0.43	3.23	0.3	NA	NA
	0 m	3.58	0.42	2.84	0.29	2.74	0.26	3.73	0.32
8 am	1 m	3.73	0.42	2.73	0.27	2.66	0.25	4.2	0.32
	1.7 m	3.64	0.4	3.02	0.28	3.16	0.27	4.11	0.33

Table A.4: Mean dissolved organic carbon (DOC) and monosaccharide (MCHO) concentrations measured between August-December at the surface, intermediate, and above sediment depths at all time points.