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Microbial bioavailability of dissolved organic carbon from leachates of freshwater autotrophs

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ABSTRACT: The concentration and bioavailability of dissolved organic carbon (DOC) released during senescence of aquatic vegetation varies among autotrophs, and the corresponding response by microbial communities depends on DOC bioavailability. To evaluate microbial response to different leachate sources, experiments that measured O_2 consumption, utilization of DOC and monosaccharides, and the specific absorbance of light at 350 nm (SUVA350) were conducted on leachates from 3 primary producers. Specific absorbance at 350 nm (SUVA350) increased in 2 of 3 experiments. Increases in SUVA350 are consistent with the preferential use of labile DOC with a lower SUVA350. However, similar patterns of monosaccharide and DOC utilization do not support the hypothesis of preferential usage of labile carbohydrates over bulk DOC by the microbial community over the 8 d study. Monosaccharides appear to represent only a portion of a larger pool of labile DOC constituents that are found in autotrophic leachates that can be taken up more rapidly than metabolized by microbial communities. O_2 consumption by the microbial community varied significantly among leachate types but was relatively high during the early phase of the experiments in all leachates. Similarly, DOC uptake was relatively rapid during the early phase of the experiments. The microbial uptake of DOC relative to the consumption of dissolved oxygen, expressed as a ratio, ranged from 2.31 to 3.95 during the initial 24 h period but approached 1:1 over the duration of the experiment. These ratios suggest that 'luxury uptake' of DOC by microbial communities might have occurred in the initial phase of the experiment.

KEY WORDS: Dissolved organic carbon · Bioavailability · Monosaccharides · Leachates · Luxury uptake · *Hydrilla verticillata* · *Potamogeton illinoensis* · *Lyngbya*

INTRODUCTION

Freshwater ecosystems contribute significantly to the global carbon cycle even though they comprise only 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 (Williamson et al. 2008, Schindler 2009). 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 affects an array of physical and chemical characteristics in the water in which it grows (Carpenter & Lodge 1986). These effects include changes in light penetration, water flow, and nutrient cycling (Madsen et al. 2001, Havens 2003) 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

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plants are alive and during senescence and death (Godshalk & Wetzel 1978, Barko et al. 1986, Mann & Wetzel 1996).

Plant leachates are utilized by bacteria and are crucial for nutrient cycling because they make nutrients and dissolved organic carbon (DOC) available to higher trophic levels by incorporating these elements into their biomass, which may then be consumed by herbivores and omnivores (Benner et al. 1986, Findlay et al. 1986). Microbial utilization of leached material can be fast and can 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 (Opsahl & Benner 1999, Stepanauskas et al. 2000, Maie et al. 2006). The purpose of this study was to determine the concentration of nutrients (NO_3, NH_4, and PO_4) and DOC released through leaching from different types of autotrophs, and to assess the bioavailability of DOC to microbial communities in a series of experiments in which natural microbial communities were exposed to leachates from the different sources. Other studies have assessed the effects that submerged aquatic vegetation, such as *Hydrilla verticillata*, can have on nutrient concentration and availability, but few have directly assessed the bioavailability of carbon to the microbial community (Barko et al. 1988, Takamura et al. 2003, Gu 2006). Monosaccharide utilization rates were measured because monosaccharides are thought to be a relatively labile form of DOC that can help to explain the differences in microbial metabolism of specific compound classes.

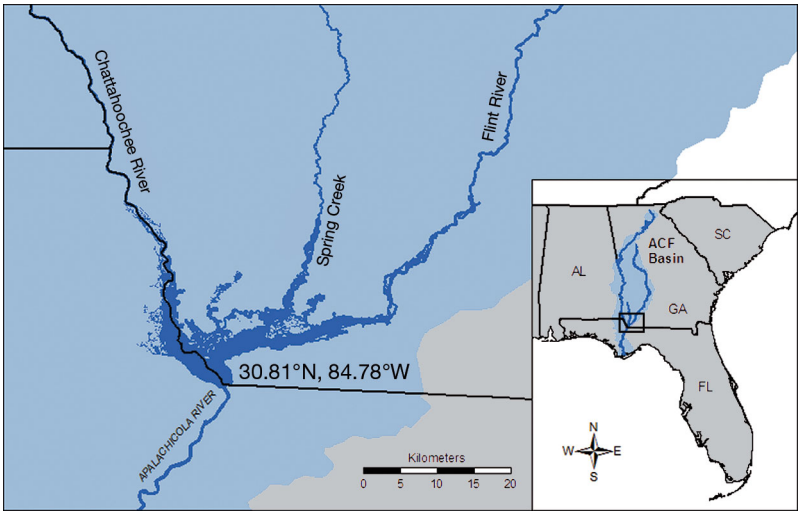

Fig. 1. Location of Lake Seminole in southwestern Georgia, USA, showing inflows from the Chattahoochee River, Flint River, and Spring Creek, and outflow to the Apalachicola River. ACF Basin: Apalachicola-Chattahoochee-Flint Basin

MATERIALS AND METHODS

Site description

Lake Seminole is a 15 216 ha impoundment located in southwestern Georgia, USA, within the Apalachicola-Chattahoochee-Flint Basin (Fig. 1). 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, the Flint River, and Spring Creek. These tributaries drain a combined land area of 46 141 km^2. Groundwater also contributes a substantial component of flow into the lake (Torak et al. 2006). Concentrations of nutrients within the lake were measured monthly over a 12 mo period during a study by McEntire (2009). Submerged aquatic vegetation has historically 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 & Maceina 2002).

Plant collection and leaching

Plant material was collected in Lake Seminole near the mouth of Spring Creek (Fig. 1). Living biomass was collected during October 2009 from Lake Seminole for the following autotrophs: hydrilla *Hydrilla verticillata*, Illinois pondweed *Potamogeton illinoensis*, and the cyanobacterial mat-forming *Lyngbya* spp. 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 h before dry weights were recorded.

Dried plant material (5 g) was placed in 1 l glass beakers to which 900 ml of ultrapure H_2O were added. The beakers were covered and refrigerated between 0 and 5°C in the dark for 14 d. 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. Filtered water (6 ml) was then added to BOD bottles to serve as a microbial inoculum. Plant leachate was added so that the DOC concentration was approximately 250 µM. Based on previous analysis of the different leachates, nutrient amendments were added in excess (0.06 M NH_4Cl, 0.06 M $C_6H_{12}O_6$, 0.06 M $Na_2HPO_4 \cdot 5H_2O$, and 0.06 M $NaNO_3$) to maintain equivalent nutrient concentrations among treatments caused by the differences in plant leachate composition. It is important to note that inorganic nutrient ratios can also alter respiratory quotients (RQs) (Cimbleris & Kalff 1998); however, nutrient amendments were added in order to focus on changes caused by differences in carbon quality. Beginning the experimental incubations with equivalent inorganic nutrient concentrations in excess enabled us to focus solely on carbon utilization without interference caused by inorganic nutrient differences. The remainder of the bottle was filled with artificial lake water (Smart & Barko 1985). All bottles were incubated in the dark for 168 h (7 d) at 27.5°C. Two controls were also included in this experiment: (1) 100% lake water; (2) 6 ml lake water, nutrient amendments, and the remainder artificial lake water. DOC and monosaccharide concentrations were measured in triplicate, and O_2 consumption was measured in duplicate. Every 24 h, 1 set of 3 bottles was removed and refrigerated below 5°C to stop microbial activity. After 168 h, contents of the bottles were filtered through 0.22 µm pre-ashed glass fiber filters to remove microbes. Samples were stored at 0 to 5°C for DOC and below 0°C for monosaccharides until analysis. Additionally, during the experiment, 2 bottles were removed every 24 h and fixed with NaI/NaOH and $MnSO_4$ for Winkler titrations (Opsahl 2005).

Sample and statistical analyses: CHN, DOC, monosaccharides, and bioassays

CHN analysis was performed on a Thermo Scientific Flash 2000 elemental analyzer. Dried plant material was ground using a Spex ball mill grinder prior to analysis. The organic carbon (OC) composition of acidified samples was determined using the method of Hedges & Stern (1984). A mass balance approach was used to evaluate whether large losses of OC occurred during leachate preparation due to microbial uptake. Initial OC in the incubations was calculated by multiplying the initial dry weight by the initial %OC. Likewise, the final OC present in the incubation was calculated by multiplying the final dry weight by the final %OC. Percent recovery was calculated by dividing the initial OC by the sum of the final OC and DOC of the leachate.

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 Myklestad method as modified by Hung & Santschi (2001). Measurements of absorbance at 595 nm for the TPTZ method were made using a Shimadzu UV-2101 spectrophotometer. Filtered water samples were also analyzed for their UV/vis absorbance spectra (280–800 nm) using the Shimadzu spectrophotometer. Specific absorbance at 350 nm (SUVA350) was calculated by dividing the absorbance at 350 nm by the concentration of DOC. Dissolved oxygen concentrations were determined through titration utilizing the Winkler method and a Mettler Toledo DL50 titrator (Pomeroy et al. 1994, Opsahl 2005). Graphic visualization of data was achieved by using Igor Pro 6.2. 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

Hydrilla verticillata plant tissue was initially composed of 25.3% OC and was composed of 34.0% OC after incubation (Table 1). *Potamogeton illinoensis* tissue contained higher levels of OC initially (38.2%) and after incubation (41.3%). *Lyngbya* spp. tissue had an initial composition of 38.2% OC and a final composition of 36.6% OC. The percent recoveries of

Table 1. Organic carbon (OC) content (±SD) and % recovery of OC before and after leaching experiments for different autotrophs

	Initial % OC	Final % OC	% Recovery
Potamogeton illinoensis	38.2 ± 0.05	41.3 ± 0.03	104.8
Hydrilla verticillata	25.3 ± 0.38	34.0 ± 0.02	117.3
Lyngbya spp.	38.2 ± 0.16	36.6 ± 0.08	101.9

OC in the *H. verticillata*, *P. illinoensis*, and *Lyngbya* spp. incubations were 117.3, 104.8, and 101.9%, respectively, indicating that substantial amounts of OC were not lost to microbial uptake during the leachate extraction.

Nutrient concentrations varied significantly among the different plant types (Table 2). *H. verticillata* leachate had the lowest concentration of DOC (1.43×10^4 µM) and the highest concentrations of PO_4 (155.8 µM) and NO_3 (4.7 µM). *Lyngbya* spp. leachate had the highest NH_4 and DOC concentrations (1.01×10^3 µM and 3.62×10^4 µM, respectively) and the lowest PO_4 and NO_3 concentrations (3.4 µM and 0.5 µM, respectively). *P. illinoensis* leachate was intermediate for all nutrient concentrations except NH_4, which was the lowest of the 3 autotrophs.

Overall, the DOC concentrations varied significantly over time among the different leachate treatments (ANOVA, $p < 0.01$; Fig. 2). The DOC concentration of BOD bottles containing *H. verticillata* leachate dropped sharply during the first 24 h (from 252.3 to 119.9 µM) and decreased at a slower rate for the next 144 h (from 119.9 to 62.4 µM). Bottles containing *Lyngbya* spp. leachate also exhibited sharply decreasing DOC concentrations during the first 24 h (from 213.1 to 58.3 µM), but DOC decreased at a slower rate for the next 144 h (from 58.3 to 25.8 µM). DOC concentrations for *P. illinoensis* bottles decreased less sharply than for *H. verticillata* or *Lyngbya* spp. for the first 24 h (from 230.6 to 156.5 µM), and they continued to decrease over the next 144 h (from 156.5 to 108.2 µM). For the lake water without added leachate, DOC decreased slightly over the duration of the experiment (from 215.6 to 190.7 µM). The samples composed of a mixture of artificial lake water and 10% lake water decreased by 3.3 µM over the first 24 h, and DOC concentrations were ~0 µM for the remainder of the experiment.

Monosaccharide concentrations varied significantly over time based upon leachate type (ANOVA, $p < 0.01$). Monosaccharide concentrations of all leachates decreased similarly over the first 24 h (1.7 µM h^{-1}) and for the remainder of the experiment (Table 3, Fig. 3). Values of the 100% lake water samples were significantly higher (40.0 µM) than those of the artificial lake water samples (5.0 µM) due to the presence of monosaccharides within the lake. Neither control group experienced significant concentration changes over the course of the experiment.

Table 2. Nutrient and dissolved organic carbon (DOC) concentrations for different autotrophs and the natural range of Lake Seminole (Georgia, USA)

	DOC (µM)	NH_4 (µM)	PO_4 (µM)	NO_3 (µM)
Potamogeton illinoensis	2.66×10^4	171.3	55.7	2.3
Hydrilla verticillata	1.43×10^4	720.7	155.8	4.7
Lyngbya spp.	3.62×10^4	1.01×10^3	3.4	0.5
Lake range	25–899.2	0–6.04	0–0.4	1.5–34.5

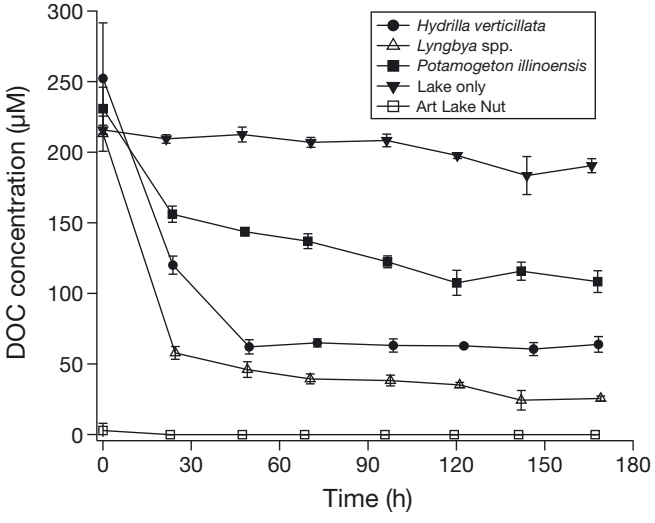

Fig. 2. Dissolved organic carbon (DOC) concentrations measured during incubations of leachates from 3 autotrophs and 2 lake water controls (Lake only: 100% lake water; Art Lake Nut: lake water + nutrient amendments + artificial lake water; see 'Materials and methods; Incubations with leachates' for details)

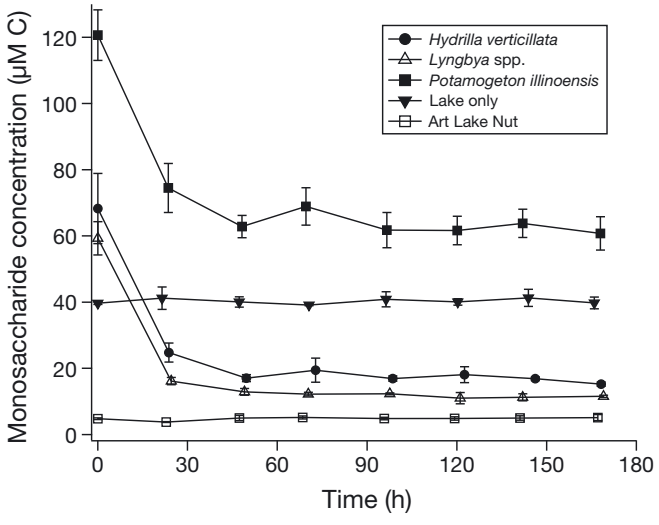

Fig. 3. Monosaccharide concentrations measured during incubations of leachates from 3 autotrophs and 2 lake water controls (Lake only: 100% lake water; Art Lake Nut: lake water + nutrient amendments + artificial lake water; see 'Materials and methods; Incubations with leachates' for details)

Table 3. Dissolved organic carbon (DOC) and monosaccharide loss rates (g C l^{-1} h^{-1}) during the first 24 h (T0–T1) and for the following 144 h (T1–TF)

	DOC		Monosaccharides	
	T0–T1	T1–TF	T0–T1	T1–TF
Potamogeton illinoensis	0.037	0.004	0.023	0.0011
Hydrilla verticillata	0.066	0.005	0.022	0.0008
Lyngbya spp.	0.078	0.003	0.022	0.0004
Lake only	0.003	0.002	0	0.0001

Total O_2 consumption was greatest in *H. verticillata* leachates. These bottles lost 57.3 µM of O_2 during the first 24 h and 189.6 µM of O_2 over 168 h (Fig. 4). *Lyngbya* spp. leachate had a more rapid loss during the first 24 h (62.8 µM of O_2 lost), but slowed considerably thereafter (152.7 µM total O_2 decrease). *P. illinoensis* leachate had the slowest O_2 consumption in the first 24 h (18.9 µM O_2) and the slowest overall O_2 consumption (106.8 µM O_2) among the 3 plant types. The O_2 consumption rates of both controls were low, but steady for the duration of the experiment.

SUVA350 varied among the samples. *P. illinoensis* leachate increased from 2.86 to 5.32 and *Lyngbya* spp. leachate increased from 0.72 to 2.72 (Fig. 5). In contrast, *H. verticillata* leachate remained fairly constant throughout the experiment with a mean of 2.01. The lake water control also remained constant during the experiment.

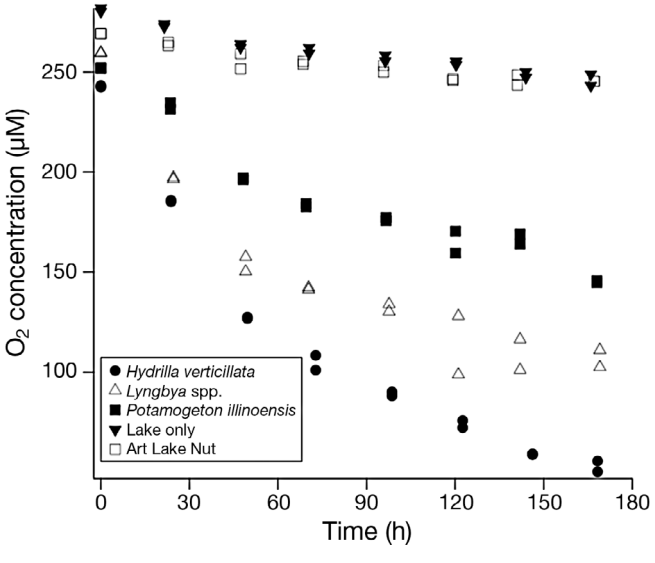

Fig. 4. O_2 concentrations measured during incubations of leachates from 3 autotrophs and 2 lake water controls (Lake only: 100% lake water; Art Lake Nut: lake water + nutrient amendments + artificial lake water; see 'Materials and methods; Incubations with leachates' for details)

The microbial uptake of DOC relative to the microbial consumption of dissolved oxygen (DOC:O_2) was calculated over the first 24 h and for the duration of the experiment using the following formulae: $(DOC_{T0} - DOC_{T1})/(DO_{T0} - DO_{T1})$ and $(DOC_{T0} - DOC_{TF})/(DO_{T0} - DO_{TF})$, respectively, where T0 = concentration at the beginning of the experiment, T1 = concentration at the first time point (24 h), and TF = concentration at the conclusion of the experiment (168 h). During the first 24 h, DOC:O_2 ratios were higher than 1:1 for the plant leachates (Fig. 6). These ratios varied from 2.31:1 for *H. verticillata* to 3.95:1 for *P. illinoensis*. The ratio of oxygen consumed to DOC consumed was approximately 1:1 over the duration of the experiment for all leachates and lake water.

DISCUSSION

OC utilization and bioavailability

Overall DOC utilization and oxygen consumption varied significantly among autotrophs, which implies differences in bioavailability to microbial communities (Figs. 2 & 4). *Hydrilla verticillata* leachate exhibited the largest decreases in O_2 concentrations. A combination of sharp decreases of DOC and monosaccharides along with increased O_2 consumption suggests that *H. verticillata* leachates contained more bioavailable carbon than the other autotrophs. SUVA350 values did not show a net increase over time (Fig. 5) as observed for *Lyngbya* spp. and *Pota-*

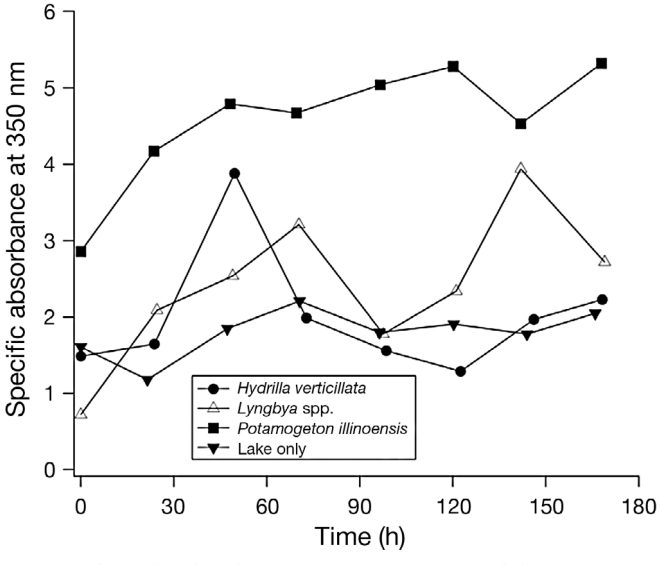

Fig. 5. Specific absorbance at 350 nm measured during incubations of leachates from 3 autotrophs and lake water only control

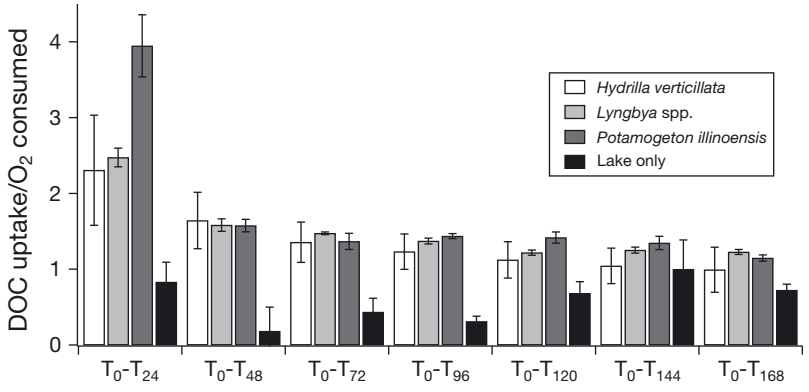

Fig. 6. Microbial uptake of dissolved organic carbon (DOC) relative to the microbial consumption of dissolved oxygen (DOC:O_2) increasing by 24 h intervals starting at time 0 and continuing through 168 h

mogeton illinoensis, which implies that refractory bulk DOC was not enriched during the experiment and further supports the greater bioavailability of carbon from *H. verticillata*. The results demonstrate that *H. verticillata* has the potential to indirectly affect nutrient cycling in Lake Seminole by causing changes in microbial metabolism, and these changes would occur on a larger scale because of the potential for high spatial coverage by this autotroph.

Lyngbya spp. leachate had the fastest O_2 consumption rates during the first 24 h, followed by decreased O_2 consumption thereafter (Fig. 4). *Lyngbya* species found in marine systems are prolific producers of secondary metabolites and other organic compounds (Nunnery et al. 2010). The patterns of DOC and monosaccharide utilization rates by *Lyngbya* spp. (Figs. 2 & 3) imply that labile carbon was present initially and was quickly taken up by microbes. These results support and further explain a previous study which demonstrated a positive correlation between cyanobacterial density and the microbial response to DOC from the cyanobacteria (Wang & Priscu 1994). Considering the rapid use of labile carbon and other organic compounds from *Lyngbya* spp. and that cyanobacterial blooms could possibly be more frequent as a result of climate change (Paerl & Huisman 2009), the potential effects of *Lyngbya* spp. on nutrient cycling observed in this study should be considered in reservoirs and lakes undergoing similar shifts in microbial community structure.

P. illinoensis incubations had the lowest rates of carbon and O_2 consumption (Figs. 2 & 4). The first 24 h were the slowest of all samples for DOC utilization, although the pattern of monosaccharide usage was comparable to the other samples. Both DOC and monosaccharide utilization rates were low after the first 24 h. These results indicate that there was a small quantity of immediately bioavailable carbon, and very little became available after the initial loss. This is supported by the lowest overall O_2 consumption rates. The structural nature of this plant could be responsible for these rates. Notably, these experiments were performed in the absence of light. Were sunlight to be included, *P. illinoensis* DOC utilization rates would likely have been enhanced because of the susceptibility of aromatic DOC to UV oxidation, which has been shown to produce highly labile DOC from biologically refractory parent molecules (Amon & Benner 1996).

Qualitatively, the leachates in this experiment were quite different and ranged in color from clear with a bluish tint (*Lyngbya* spp.) to dark tea-colored (*P. illinoensis*). This coloration implies that these leachates are optically different and that, as a result, they could have different degrees of reactivity (Kowalczuk et al. 2003, Boyd & Osburn 2004, Coble 2007). *P. illinoensis* is characterized by long stems that have fewer leaves than other submerged vegetation. It is likely that more structural components, such as aromatic rings, are present in these leachates than in the others. The initially high SUVA350 (Fig. 5) and stained, tea-like appearance of this leachate provides evidence for a larger aromatic component, which may explain the observed slower rates of metabolism.

There were also striking similarities among autotrophs with regard to monosaccharide uptake during the first 24 h as evidenced by comparable rates of monosaccharide loss (Table 3). These loss rates demonstrate that monosaccharides were readily available to the microbial community. Loss rate patterns for DOC in the initial 24 h were also similar for all autotrophs, indicating that a portion of the bulk DOC was available. However, *P. illinoensis* loss rates were lower than those of the other autotrophs. The structural nature of *P. illinoensis* might require further conditioning to make the DOC more available for use, i.e. increased UV photooxidation. In addition to *P. illinoensis* leachate, all leachates and the lake water control had a portion of the monosaccharides that were not used by the microbial community. It is possible that the structural composition of these monosaccharides prevented uptake and utilization.

We found that the DOC:O_2 ratios for all plant leachates for the first time period were greater than 2:1 while remaining near 1:1 for the overall experi-

ment (Fig. 6). The initially elevated DOC:O_2 ratios suggest that more OC is being taken up by microbes than is being concurrently respired and may represent 'luxury uptake' by the microbial community. This concept of luxury uptake of carbon has been reported for bacteria in marine systems (Kuipers et al. 2000) but not in freshwater systems. A separate component of our project (Shivers 2010) demonstrated OC limitations in the microbial communities present in the study area by measuring O_2 consumption after adding a series of nutrients and a source of labile carbon in bioassay experiments. Similar carbon limitation has also been demonstrated in marine systems (Carlson & Ducklow 1996). Luxury uptake is a plausible mechanism to provide microbial communities the ability to rapidly take up dissolved OC that may be the primary substrate limiting growth.

Previous studies have demonstrated that bacteria store carbon as reserve energy sources through the synthesis of storage molecules, such as polysaccharides and lipids, particularly through the synthesis of polyhydroxyalkanoates (PHAs) (Wilkinson 1963, Anderson & Dawes 1990). Poly(3-hydroxybutyrate) is one of the most abundant and well-studied PHAs and has been found in a variety of bacterial species. It is possible that the storage molecules were synthesized from the excess DOC uptake and stored for future use, supporting the concept of luxury uptake in the presence of labile DOC. Alternatively, it is possible that the microbial populations immediately metabolized the labile DOC and increased the total microbial biomass and thus the total particulate OC fraction in the experiment. Neither specific metabolites, such as PHAs, nor estimates of microbial biomass were included in this study, and the relative roles that these processes may play in explaining the early rapid uptake of DOC are as yet unknown. The focus of this study was on DOC, and other fractions of carbon were unfortunately not analyzed; thus, elucidating the exact mechanism falls outside the scope of this study.

An alternative explanation for the differences in DOC:O_2 ratios is preferential metabolism of more oxidized constituents which could also explain increases in these ratios. Studies have demonstrated that leachate and exudates from *H. verticillata* contain organic acids, such as caffeic acid ester and other phenolic compounds (Glomski et al. 2002, Gao et al. 2011, Wang et al. 2015). These compounds have been shown to increase RQs as the microbial community preferentially uses organic acids instead of more reduced substrates (Berggren et al. 2012). It is likely that freshwater *Lyngbya* species also produce an array of organic compounds that could be used preferentially by the microbial community producing a greater RQ. Wang et al. (2010) found that extracts of 2 *Potamogeton* species contained strong, polar organic acids that were mostly different than those found in *H. verticillata*. These extracts contained palmitic acid, which has been shown to lower the RQ to <1.

In order for the changes in RQs to account for the elevated DOC:O_2 ratios, the leachates would need to consist of a large proportion of reduced constituents. As indicated above, prior studies have indicated that reduced compounds are present within these plant leachates, but it is not clear whether the proportions are high enough to drive changes in RQs. The leachates used in this study were shown to contain high proportions of monosaccharides, consistent with the results of Hung et al. (2005) in a freshwater system in Texas, USA. Metabolism of carbohydrates would be expected to have an RQ close to 1 and argues in favor of the possibility that the increased DOC:O_2 resulted from luxury uptake. However, it is also possible that the remaining labile DOC contains a substantial proportion of more reduced compounds and RQs are considerably different during the rapid metabolism of labile DOC derived from plant leachates. A comprehensive characterization of labile DOC at the molecular level would resolve these possibilities, but, to date, only a small portion of the total DOC in leachates has been characterized at the molecular level (Maie et al. 2006).

Nutrient composition 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 & Vitousek 1998). Quantitatively, the nutrient compositions of the leachates were variable, although all concentrations were significantly higher than ambient concentrations within the lake. This variation could be expected considering the different physiologies of these macrophytes. The different nutrient compositions and when those nutrients are released can have an impact on nutrient cycling in the surrounding waters (Carpenter 1980, Carpenter & Lodge 1986). Because *H. verticillata* can cover large spatial areas and has the potential to leach high concentrations of NO_3 (4.7 µM), PO_4 (155.8 µM), and NH_4 (720.7 µM), 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* spp. *Lyngbya* spp. releases high concentrations of NH_4 (1.01×10^3 µM) upon senescence and could be responsible for nitrogen transport throughout a water body. Additionally, *Lyngbya* spp. can fix nitrogen under anaerobic conditions in the benthos (Phlips et al. 1992). Therefore, the nutrient composition of primary producers can affect shallow areas where macrophytes are more abundant, but also cycling throughout the lake.

The availability of labile carbon is important because it serves as an energy source for the microbial community. Our study provides evidence that autotrophs release a different quantity and quality of labile carbon upon senescence and that microbial communities can respond to these differences. One possibility is that luxury uptake of labile carbon is occurring and serves as an effective mechanism for microbial communities to respond to the release of this substrate by autotrophs. In addition to a large proportion of labile monosaccharides, labile DOC contains an abundance of as yet unidentified compounds. Labile carbon sources increase microbial metabolism, which directly impacts nutrient cycling within aquatic systems. Understanding differences in bioavailability of carbon is therefore crucial to understanding nutrient cycling in aquatic ecosystems.

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