EFFECTS OF ORGANIC MATTER PROCESSING ON OXYGEN DEMAND IN A SOUTH GEORGIA BLACKWATER RIVER

by

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(Under the Direction of George Vellidis and Catherine M. Pringle)

ABSTRACT

Many rivers periodically experience system-wide hypoxia, sometimes without significant nutrient loading. The primary goal of a long-term project titled "Dissolved Oxygen Dynamics in the Upper Suwannee River Basin" was to determine factors (natural or anthropogenic) contributing to low dissolved oxygen concentrations observed in Georgia's coastal plain blackwater rivers. As a project component, this study assessed effects of organic matter processing on oxygen demand in third-order-stream and fifth-order-swamp reaches of southern Georgia's Little River. Differences in microbial biomass explained variation in leaf litter oxygen and nutrient uptake among tree species, but nutrient uptake was additionally affected by aluminum and iron accumulating in litter. Microbial biomass was negatively correlated to litter chemistry parameters such as the lignocellulose index, and while labile litter supports higher microbial oxygen uptake, it is also preferentially colonized by macroinvertebrates and breaks down more rapidly. Therefore, recalcitrant litter may make greater long-term contributions to oxygen demand. Detrital standing stocks consisted primarily of leaf litter, but bald cypress (*Taxodium distichum*) produced significantly more litter per trunk biomass relative to other species in the swamp. Estimates of leaf litter microbial respiration (5.54 g O_2 $m^{-2} \cdot day^{-1}$)

accounted for 89% of sediment oxygen demand (SOD) measured by other researchers (6.20 g O_2 $m^{-2} \cdot day^{-1}$) in the same river, illustrating the importance of leaf litter breakdown to overall SOD. Although breakdown rates were faster in the swamp than in the stream, a greater percentage of initial leaf litter standing stocks were retained (54% vs. 30%) in the swamp, potentially due to lower water velocity. Over a seven-year study period, dissolved organic carbon (DOC) transport decreased substantially as annual dry period length increased, while DOC concentration and mineralization were enhanced at low flows. Although DOC mineralization was a small source of oxygen demand (roughly 4%) compared to leaf litter breakdown, our findings suggest that as droughts intensify, temperatures rise, and discharge decreases, enhanced DOC mineralization and reduced downstream DOC export may occur. Research presented here demonstrates that leaf litter accounts for a large natural source of oxygen demand, and illustrates the complex interactions affecting organic matter processing in a Georgia coastal plain blackwater river.

INDEX WORDS: Aluminum, Bacteria, Cellulose, Dissolved organic carbon, Dissolved oxygen, DO, DOC, EEM, Fungi, Hypoxia, Iron, PARAFAC, Respiration, Lignin, Lignocellulose index, Manganese, Nutrients, *Nyssa*, *Taxodium*

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DEDICATION

To my mom and dad, who shared their love of nature with me and influenced my life more than they'll ever know.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Dissolved oxygen (DO) availability is a critical factor determining the structure and function of aquatic ecosystems. It determines rates and dominant pathways of nitrogen (N) cycling (Kemp and Dodds 2002); fluxes of phosphorus (P), manganese (Mn), iron (Fe), Cobalt (Co), and dissolved organic carbon (DOC) between sediments and overlying water (Sundby et al. 1986, Skoog and Arias-Esquivel 2009). In addition, oxygen availability also shapes the distribution and survival of organisms at both microscopic and ecosystem scales (Malard and Hervant 1999, Breitburg et al. 2003, Spieles and Mitsch 2003), and has a strong influence on trophic interactions (Breitburg et al. 1997, Moore and Townsend 1998, Decker et al. 2004). DO concentrations can reach low levels through anthropogenic nutrient loading, which causes the overgrowth and subsequent breakdown of algal blooms and enhancement of microbial respiration (Schindler 1971, Carpenter et al. 1998). Specific habitats in freshwater ecosystems may experience hypoxia or anoxia during certain times of the year, such as the bottom waters of stratified lakes (Wetzel 2001), or hyporheic zones of streams and rivers (Malard and Hervant 1999), but hypoxia throughout the water column of streams and rivers is far less common, outside of cases involving anthropogenic nutrient loading (Mallin et al. 2006). However, lotic ecosystems may experience system-wide hypoxia for extended periods of time each year, apparently in the absence significant nutrient loading. Large wetlands such as the Pantanal (Hamilton et al. 1997), many estuaries (Pomeroy and Cai 2006), forested temporary ponds (Rubbo et al. 2006), and blackwater rivers (Meyer 1992) are aquatic ecosystems that experience

"natural" periods of low dissolved oxygen concentrations during certain times of year. For example, blackwater rivers experience high temperatures, low discharge, and low DO during summer months (Mulholland 1981, Meyer 1992).

Many of the blackwater rivers in North America's southeastern coastal plain exist in areas dominated by agriculture and silviculture (Brown et al. 2005), and although eutrophication can cause hypoxia in these systems (McCormick and Laing 2003, Mallin et al. 2004), many that are low in oxygen do not show obvious signs of nutrient loading (Meyer 1992, Carey et al. 2007, Todd et al. 2009). It has been hypothesized that a combination of high temperatures and large stocks of organic matter contribute to low DO in coastal plain blackwater streams with otherwise good water quality (Vellidis et al. 2003b). Nevertheless, when DO concentrations in a waterbody segment drops below government-mandated levels, the development of Total Maximum Daily Load (TMDL) management and implementation plans becomes necessary. The TMDL plan determines the total amount of pollutant that a waterbody segment can receive while still maintaining water quality standards, and also allocates pollutant waste loads to specific point and non-point sources.

Although TMDL plans are important for the protection of water quality, legal approaches involved in implementation may oversimplify complex biological processes which occur naturally in these systems, leading to citation of water bodies with low DO resulting from natural causes (Pomeroy and Cai 2006). In forested streams of the coastal plain, low elevational gradients reduce water velocity and reaeration rates, high water temperatures reduce the oxygen-holding capacity of water, and dense forest canopies produce large inputs of organic matter while simultaneously reducing light penetration and limiting aquatic primary productivity (Carey et al. 2007). These factors may contribute to low DO in the absence of nutrient loading. For example,

in a survey of 43 "least-impaired" forest streams in Louisiana, Ice and Sugden (2003) found that 80% of reaches had DO concentrations below 5 mg L^{-1} (Louisiana's standard for impairment), and almost 60% of reaches displayed concentrations below 3 mg L^{-1}. Low flow and high concentrations of substrate organic matter were implicated as a probable cause. Likewise, Hampson (1989) surveyed a forested (77%) wetlands stream basin in central Florida, and found that it repeatedly violated Florida government standards for DO (5 mg L^{-1}) over a five year study period. High water temperatures and natural oxygen-demanding substances (e.g., organic matter) were cited as the major factors affecting DO concentrations. Finally, Slack and Feltz (1968) found an inverse relationship between leaf litter inputs, and pH and DO in a Virginia piedmont stream.

The Suwannee River has relatively low human impacts (Bricker et al. 1999), as no major impoundments have been constructed within its drainage basin (Katz and Raabe 2005). Despite this fact, portions of the Suwannee River basin do not meet government-mandated standards for several water quality parameters, including DO concentrations. For example, the Georgia Department of Natural Resources Environmental Protection Division (DNR-EPD) mandates that streams must maintain an average DO concentration of 5 mg L^{-1} and a minimum of 4 mg L^{-1}, but DO concentrations in the Little River of the Suwannee River basin regularly reach levels below 1 mg L^{-1} during summer months (Carey et al. 2007, Utley et al. 2008, Todd et al. 2009). Low DO is a common feature of coastal plain blackwater rivers (Meyer 1992), and 61 of the 67 water body segments cited under the federal Clean Water Act's 2001 303(d) list in the Coastal Plain of Georgia violated standards for DO (Vellidis et al. 2003b). While it is known that DO concentrations below government standards exist within the Suwannee River basin, the factors influencing the oxygen demand have not been fully explored. Recognizing that an oxygen

minima of 4 mg L^{-1} may be unrealistically high, the Georgia DNR-EPD has proposed a new DO standard of 90% of natural DO during critical flow conditions. However, little information is available to establish what natural DO concentrations within these systems should be. The western upper Suwannee River basin includes the Little, Withlacoochee, and Alapaha River watersheds. Land use within all three watersheds is primarily agricultural (GADNR 2002), so there is good reason to suspect low DO to be due in part to nutrient runoff in some river sections within the basin. However, rivers within many of these watersheds are surrounded by large riparian buffers, which may effectively filter and retain nutrients before they can be transported to the river channel (Lowrance et al. 1984). Carey et al. (2007) demonstrated that algal growth within the Little River may be primarily shade limited, and secondarily nutrient limited (P or N+P) in late spring.

Areal microbial respiration rates are thought to be naturally high in coastal plain rivers due to the availability of large quantities of organic matter (Meyer 1992), resulting from high leaf litter inputs from riparian and riverine trees (Cuffney 1988). As a consequence, leaf litter breakdown and concomitant microbial oxygen demands may be responsible for the seasonal DO dynamics in these rivers. Detritus is an important source of energy in forested streams (Wallace et al. 1997) and riparian trees generally dominate terrestrial inputs to stream ecosystems (Webster et al. 1999). In some aquatic ecosystems, detritus may also affect ecosystem function by depleting oxygen (Wetzel 2001). Low air and water temperatures, high re-aeration rates due to steep gradients, and evolution of oxygen by algae and aquatic plants are common sources of oxygen in aquatic ecosystems (Wetzel 2001), but these factors are often reduced or absent in forested blackwater streams in the coastal plain. Decomposing organic matter can be a major sink for oxygen in aquatic ecosystems (Bosserman 1984, Hamilton et al. 1997), and benthic

oxygen demand is often correlated with the amount of organic matter found in the sediment (Fuss and Smock 1996, Todd et al. 2010). Microbial respiration rates per gram of leaf litter are often higher than rates associated with of other forms of organic matter (wood, surface and hyporheic sediments) in the substratum of blackwater rivers (Smock 1997). Although total standing stocks of wood may be higher in blackwater rivers (Meyer et al. 1997, Smock 1997), yearly inputs of leaves are significant (Mulholland 1981, Cuffney 1988) and far outweigh inputs of wood. Higher turnover rates have been measured for leaves and fine particulate organic matter (FPOM) than for wood in temperate streams (Webster et al. 1999), and therefore tree leaf litter and FPOM may generate a larger percentage of system-wide oxygen demand by promoting higher microbial activity.

Different tree species produce leaves that vary in their initial content of nutrients (nitrogen, phosphorus) and structural compounds such as lignin, cellulose, and hemicellulose. Once leaves enter into streams and rivers, leaf litter of higher initial nutrient content often promotes enhanced microbial growth, relative to litter that is lower in nutrient content. While cellulose and hemicellulose (collectively referred to as holocellulose) are readily decomposed by microorganisms, lignin is more recalcitrant and resists microbial degradation. Furthermore, lignin can only be broken down in oxygenated environments, and therefore may be especially limiting for microbial utilization and growth under hypoxic conditions, such as those encountered in blackwater rivers. The fact that differences among tree species in leaf chemical composition results in differences in microbial biomass and respiration provides a potential link between forest composition and oxygen demand in forested aquatic ecosystems.

Forest composition may shift temporally and spatially in blackwater swamps and rivers due to hydrological differences among river reaches (Burke et al. 2003), natural disturbance and

succession (Penfound 1952), and human impacts such as logging. Ellison et al. (2005) list the bald cypress (*Taxodium distichum*), a dominant species in many coastal plain blackwater rivers, as one of the world's "foundation species" that is being lost due to intensive logging and removal. In the Okefenokee Swamp, for example, the majority of logging operations that occurred in the early 1900s were in stands of mature cypress (Hopkins 1947), but most of the harvested areas in the Okefenokee Swamp show no sign of returning to their pre-logging species composition (Hamilton 1984), and are now dominated by swamp tupelo (*Nyssa biflora*) or bay magnolia (*Magnolia virginiana*). Patterns of secondary succession of trees in blackwater rivers may vary significantly depending on hydrology (Hodges 1997), and other studies in Florida have demonstrated post-logging shifts to mixed hardwoods or willow (*Salix* sp.) (Gunderson 1984), or to ash (*Fraxinus* sp.) (Wharton et al. 1977).

Leaf litter species may differ in their effects on DO through enhancement or depression of microbial biomass and respiration (i.e., oxygen demand) associated with coarse particulate organic matter. Furthermore, leaf litter may also have far-reaching effects on stream ecosystem metabolism as microbes downstream utilize dissolved organic carbon (DOC) released from decomposing leaves (Meyer et al. 1987, Meyer et al. 1998). Blackwater rivers are named for their high concentrations of DOC (Sabater et al. 1993). As leaf litter decomposes in aquatic ecosystems, it releases soluble organic compounds that can provide large amounts of DOC. Supply and bioavailability of DOC may alter microbial community structure as well as ecosystem processes such as microbial respiration (Findlay et al. 2003). Leaves release considerably more DOC than wood (O'Connell et al. 2000), indicating that leaf litter may play a strong functional role in river DOC dynamics. Transport of dissolved organic carbon makes up (by far) the largest carbon export in most blackwater rivers (Mulholland 1981, Meyer et al. 1997,

Smock 1997). However, a large portion of this DOC may be refractory and not readily available to microbes (Sun et al. 1997). DOC in blackwater river systems may become increasingly refractory with age and distance downstream (Leff and Meyer 1991), although it has been suggested that relatively refractory humic substances may play a significant role in ecosystem metabolism through photolysis to simple compounds by ultraviolet radiation (Wetzel et al. 1995). DOC leachates from freshly-abscised leaf litter may enhance (Mills et al. 1995, Koetsier et al. 1997, Strauss and Lamberti 2002) or, more infrequently, inhibit (McArthur et al. 1994) various aspects of the microbial community. Leaf litter species are known to differ in the relative amounts of total DOC released (Mills et al. 1995), rapidly leaching DOC within 1-2 weeks of submergence (Webster and Benfield 1986). Although watershed processes may be more important than in-stream processes in controlling DOC in streams (Mulholland 1997), leaf litter in the stream channel has been estimated to contribute nearly one third of DOC exports in some forested streams (Meyer et al. 1998).

The determination of "natural" DO concentrations in blackwater rivers requires a larger number of datasets on organic matter breakdown and respiration dynamics than the few that are currently available. The primary goal of a long-term interdisciplinary project titled "Dissolved Oxygen Dynamics in the Upper Suwannee River Basin" (often referred to as the "Low Dissolved Oxygen Project") was to determine factors (natural or anthropogenic) contributing to the low dissolved oxygen concentrations commonly observed in Georgia's coastal plain blackwater rivers. The project is a collaborative effort between agricultural scientists from the University of Georgia, the United States Department of Agriculture, and the Georgia DNR-EPD to understand the complex processes that determine DO levels in coastal plain blackwater rivers. The primary goal of my dissertation research was to address this objective with a series of novel ecological

studies, while simultaneously providing the data that policy makers require in order to make informed decisions about new state water quality guidelines. The Georgia DNR-EPD and consultants from Tetratech, Inc. use the Environmental Fluid Dynamics Code (EFDC) model and field data to develop target DO concentrations for each river that fails to meet current guidelines. Model parameters include temperature, dissolved organic carbon (DOC) and nutrient levels, which are relatively easy to collect. Measuring benthic oxygen demand from decomposing organic matter, however, is labor-intensive and rarely measured directly, especially in large rivers. Therefore, benthic oxygen demand is a major area of model uncertainty. It can be estimated from several parameters including litter inputs and breakdown rates, but these data are also scarce for blackwater rivers. Currently, organic matter flux and processing rate parameterization data from the Ogeechee River (Meyer et al 1997) are applied to all blackwater rivers in southern Georgia. Although a comprehensive study of organic matter dynamics within the Ogeechee, these data do not represent the variable conditions among blackwater rivers. Parameters such as discharge, DOC and nutrient concentrations may vary significantly among rivers and river segments within a river, resulting in inaccurate estimates of benthic oxygen demand and target DO minima. DOC concentration and leaf litter inputs and breakdown rates are critical components of the EFDC model being used to address low DO issues. Therefore, my study examines organic matter processing, and specifically focuses on leaf litter breakdown and DOC cycling within the Little River.

CHAPTER 2

LEAF LITTER NUTRIENT UPTAKE IN BLACKWATER RIVERS: INFLUENCE OF BIOTIC AND ABIOTIC DRIVERS

[1]A.S. Mehring, K.A. Kuehn, A. Thompson, G. Vellidis, C.M. Pringle, A.D. Rosemond, M.R. First, and R.R. Lowrance. To be submitted to *Functional Ecology*.

Abstract

Leaf litter may sequester nutrients as it breaks down in streams, gradually accumulating greater total masses of nitrogen (N) and phosphorus (P) than were present upon initial submergence. N and P uptake can be affected by nutrients associated with microbial biomass, decaying plant or animal tissue, and accumulated metal oxides, which have a concomitant influence on nutrient cycling and detrital quality as a basal resource. We examined the relative importance of these various biotic and abiotic modes of nutrient immobilization in decaying leaf litter. Leaf packs of tupelo (*Nyssa ogeche*), maple (*Acer rubrum*), and oak (*Quercus nigra*) were incubated in an intermittent blackwater stream and periodically analyzed for mass loss, changes in structural chemistry, litter nutrient and metal content, and microbial biomass and activity over a 431-day incubation. These data were used to construct candidate models explaining temporal changes in detrital nutrient content in three species of leaf litter. Additional models comparing estimated nutrient stocks (sum of fungal, bacterial, and labile and recalcitrant leaf tissue) to observed total detrital nutrient stocks were generated using a Monte Carlo simulation. Labile tupelo litter had the highest N and P content throughout the study. However, slower mass loss of oak litter facilitated greater nutrient retention over longer incubations, suggesting that recalcitrant litter may serve as an important long-term sink for nutrients. Microbial biomass and metal content were important parameters explaining litter nutrient content throughout short incubations (≤75 days), with slightly greater weight of evidence for metal content as an important correlate of nutrient content over longer incubations (>430 days). While fungal biomass became more similar among litter species after long incubation times, differences in bacterial biomass and metal and nutrient content persisted, suggesting an important role of surficial biofilms in nutrient uptake. Results of Monte Carlo analysis additionally demonstrated that while allowing for a great

deal of flexibility in leaf litter nutrient leaching rates, concentration of N in structural tissues, and in microbial stoichiometric nutrient ratios, a portion of detrital nutrients could not be accounted for by biotic drivers, and instead may have been influenced by abiotic drivers. Abiotic nutrient pools, such as nutrients bound to sediments and associated metals on detritus surfaces, may be recycled at different rates than nutrients incorporated into microbial biomass. Assessing the relative influence of these mechanisms holds promise for advancing our understanding of detrital nutrient uptake in blackwater streams and rivers.

Introduction

Anthropogenic nutrient loading is assumed to be low in many of the blackwater rivers draining Georgia's coastal plain, primarily because of low water column nitrogen (N) and phosphorus (P) concentrations. Carey et al. (2007) demonstrated that periphyton growth in the Little River was primarily shade limited and secondarily nutrient limited during late spring and early summer. However, inference of trophic status based on these types of data may be somewhat misleading, if there is not a complete understanding of nutrient loading and uptake rates within the system (Dodds 2003). For example, leaf litter may sequester nutrients from the water column, gradually accumulating a greater total mass of N or P than was present upon initial submergence (Suberkropp et al. 1976, Meyer 1980). Therefore, rivers receiving large quantities of organic matter from riparian forests may be capable of considerable nutrient uptake (Brinson 1977, Mulholland 2004). Nutrients taken up and stored in leaf litter or sediments may not be readily detected in the water column, nor would they be readily available for enhancement of algal growth, but they could still contribute substantially to oxygen demand in blackwater rivers through enhancement of benthic microbial biomass and oxygen uptake (Mallin et al.

2004). A more complete understanding of the degree to which nutrients are sequestered by leaf litter in blackwater rivers could better inform water policy, especially when assumptions of low anthropogenic impact are based primarily on water column nutrient concentrations.

Multiple pathways of nutrient uptake exist in leaf litter, and N and P content may be influenced simultaneously by several biotic and abiotic pools, such as the nutrient content and structural chemistry of decaying plant tissue (Moore et al. 2004), the prevailing microbial community (Makino et al. 2003), and accumulated inorganic matter (Meyer 1980). Nutrient uptake by litter-inhabiting fungi and bacteria and inorganic nutrient adsorption may lead to net nutrient sequestration, and therefore limit downstream transport of nutrients from their origin (Peterson et al. 2001, Mulholland 2004, Bernot and Dodds 2005). However, the overall ability of leaf litter to serve as a sink for nutrients also depends on its rate of decomposition (i.e., mass loss). While labile litter usually supports higher biomass of microorganisms and therefore greater microbial uptake and retention of nutrients, the litter itself is lost more rapidly from the system via microbial breakdown and mineralization as well as invertebrate feeding. The degree to which these two opposing forces balance each other is unclear.

Inorganic particles from sediments or *de novo* precipitates of iron (Fe) and aluminum (Al) oxides become incorporated into the detrital matrix during breakdown (Meyer 1980, Cameron and Spencer 1989, Chamier et al. 1989), and may impact detrital nutrient dynamics as well. It is well established that P forms strong inner-sphere bonds with cations such as Al, Fe, calcium (Ca), and manganese (Mn) (Sigg and Stumm 1981, Hesterberg et al. 2011), which comprise the majority of surface cations on sediment particles in aquatic ecosystems (Richardson and Vaithiyanathan 2009). Similar interactions exist for nitrogen, as both ammonium ($NH4^{+}$) and

dissolved inorganic nitrogen (DON) strongly partition to clay particle surfaces (Triska et al. 1994, Aufdenkampe et al. 2001).

Each nutrient pool's relative influence on detrital nutrient content changes at a different rate, and is regulated by processes including: 1) nutrient loss through leaching and decay (mineralization) of labile and recalcitrant components of plant tissue (Ibrahima et al. 1995), 2) nutrient immobilization through microbial colonization and biomass accrual (Cross et al. 2005, Cleveland and Liptzin 2007), 3) accumulation of inorganic sediments and associated complexation of nutrients. Additionally, relatively recalcitrant pools of N may develop in litter over time. Phenols and lignin form recalcitrant complexes with plant proteins and nitrogen-containing microbial exoenzymes (Suberkropp et al. 1976, Schlesinger and Hasey 1981), potentially creating pools of N that are sequestered and released slowly. Chitin in fungal tissue constitutes an another N-containing pool that may not decompose rapidly (Gleixner et al. 2002). Determining the relative contributions of biotic and abiotic nutrient pools will aid in a better understanding of leaf litter nutrient cycling, as nutrients bound to metals may not be as bioavailable as those associated with microbial biomass.

To address these questions, we examined breakdown, litter structural chemistry, fungal and bacterial biomass, and nutrient and metal immobilization associated with three different leaf litter species in an intermittent blackwater stream. This incubation period spanned more than one year and included a period of complete natural drying of the stream channel. Measured components of nutrients were incorporated into multiple regression model comparisons as well as Monte Carlo simulation models, in order to estimate relative nutrient contributions from the various biotic and abiotic pools. The main objectives of the study were to determine: (1) how different leaf litter species contribute to nutrient uptake and retention and metal oxide

accumulation; (2) the relationship of biotic (fungi and bacteria) and abiotic (accumulation of metal oxides) factors to nutrient uptake; and (3) whether interspecies differences in the ability to serve as nutrient sinks are maintained over long periods of time. We predicted that: (1) nutrient content and retention and metal adsorption would differ among leaf litter species; (2) both biotic and abiotic factors would explain differences in nutrient uptake; and (3) that interspecies differences would eventually become more similar over time.

Methods

Study site – This study was conducted in a heavily forested third-order reach of the Little River, a blackwater river in Turner County, Georgia, USA, draining the Atlantic coastal plain of North America. The study stream is contained within the Little River Experimental Watershed (LREW). Located in the headwaters of the upper Suwannee River basin, the 33,400-ha LREW was equipped in the 1960's and 1970's for continuous rainfall and stream flow measurements by the Southeast Watershed Research Laboratory (SEWRL) of the United States Department of Agriculture's Agricultural Research Service (USDA-ARS). Currently, the LREW has seven nested gauged sub-watersheds ranging in size from 260 to 11,500 ha. Detailed records of stream flow, nutrient concentrations, and dissolved oxygen (DO) concentrations are regularly collected in each sub-watershed (hourly, daily or weekly depending on sub-watershed and type of data collected). The study reach (31º41'32"N, 83º42'09"W) drains a 2,200 ha catchment, and meanders through a second-growth forest floodplain with variable discharge and long periods during the summer and fall months when the stream channel dries completely (Figure 2.1). Clay-containing soils known to be rich in metals (Lowrance and Vellidis 1995) (Appendix A) are prevalent throughout the region, with water column concentrations of iron and manganese in

downstream reaches of the Little River ranging 0.039-1.92 and 0.0056-1.72 mg L^{-1}, respectively (unpublished data). Other chemical and physical characteristics of the study reach are summarized in Table 2.1.

Field procedures – We examined the breakdown, nutrient and metal content, and microbial dynamics associated with decomposing leaf litter of three common southeastern coastal plain tree species that differ in their initial litter chemistry (Table 2.2). Leaf litter with lowest initial concentrations of lignin and cellulose, highest initial concentrations of N and P, and lowest lignin:N ratios were considered the most labile. The three species selected, in order from most recalcitrant to most labile, were water oak (*Quercus nigra* L., hereafter referred to as "oak"), trident red maple (*Acer rubrum* var. trilobum Torr. & Gray ex K. Koch, hereafter referred to as "maple"), and Ogeechee tupelo (*Nyssa ogeche* Bartram ex Marsh, hereafter referred to as "tupelo"). The three litter species also differed in surface roughness: leaves of oak are mostly smooth on upper and lower surfaces (Brown and Kirkman 2000); leaves of maple are pubescent below (Bicknell 1913); and tupelo's leaves are "velvety hairy" (Duncan and Duncan 1988). Between 2006 and 2007, 10-gram single-species leaf litter bags were incubated in the stream reach. Leaf litter from each species was collected immediately after abscission, air-dried in the laboratory, and placed into plastic coarse mesh pecan bags (19 × 38 cm, 25 mm^2 mesh; Cady Industries Inc., Georgia) following methods described by (Benfield 1996). Leaf litter bags were deployed in study reaches and were grouped in arrays affixed to PVC tubing within the basin of the stream channel. Each array consisted of three leaf litter bags, with each bag containing litter from a different tree species. Leaf litter bags were organized into a randomized complete block design, with arrays grouped into blocks based on longitudinal distance

downstream in the stream channel. Five bags of each leaf litter species treatment (one from each block) were removed from the stream on each sampling date.

In situ rates of microbial respiration were estimated from DO uptake using methods described by Suberkropp et al. (2010). Briefly, after a leaf pack was removed from the stream, ten 17-mm-diameter disks were cut from a single species and immediately enclosed into a 26-mL respiration chamber containing unfiltered stream water. Changes in DO concentrations within chambers were measured every 5 minutes for 30 minutes using a YSI 5100 Dissolved Oxygen Meter (Yellow Springs, OH, U.S.A.). All measurements were conducted at ambient stream water temperatures in darkness. Oxygen uptake rate was determined by the slope of the regression of DO concentrations versus time, minus a control slope that measured stream water alone. Following respiration measurements, leaf discs were placed into labeled foil packets, placed on ice and transported to the laboratory.

Additional leaf discs were simultaneously cut from collected litter material and preserved in the field for estimation of fungal (ergosterol) and bacterial biomass. Leaf disks for fungal biomass (five 12-mm-diameter disks) were placed into clean 20-ml plastic scintillation vials and preserved with 5 ml HPLC-grade methanol. Leaf disks for bacterial biomass (five 4-mm-diameter disks) were placed into sterile 15-ml polystyrene tubes containing 5 ml sterile-filtered 2% phosphate buffered formalin. All samples were immediately placed on ice and transported to the laboratory where they were stored in the dark at -20ºC (fungal biomass) and 4°C (bacterial biomass) until analyzed. The remaining litter bag material was placed into clean, individually-labeled resealable plastic bags filled with stream water, and immediately placed on ice and transported to the laboratory for further processing.

Laboratory procedures – In the laboratory, leaf disks from respiration measurements were oven-dried for one week at 60ºC, weighed, and a sub-sample combusted at 500ºC to determine leaf litter ash-free dry mass (AFDM). The remaining leaf material within litter bags was rinsed over a sieve (1 mm mesh size) to remove macroinvertebrates and other foreign material. Leaves were dried for one week at 60ºC, weighed, and a sub-sample combusted at 500ºC to determine AFDM. Breakdown rate (k) was determined from the slope of the natural log of mass remaining versus time in days (Webster and Benfield 1986).

Litter chemistry was analyzed with remaining oven-dried leaf material from litterbags. Litter was ground to a powder (250-µm mesh), and C and N concentrations analyzed using a Carlo Erba 1500N CHN Analyzer (Carlo Erba, Milan, Italy). Cellulose, hemicellulose, and lignin concentrations were determined using an Ankom A200 Fiber Analyzer (Ankom, Macedon, New York, USA). To analyze changes in phosphorus and metal (aluminum, iron, and manganese) content of litter through time, 10 mg of ground dried litter was weighed, combusted at 500°C, extracted with 0.25 mL of aqua regia, and diluted with 10 mL of deionzied water. Phosphorus was measured from diluted extracts on a colorimetric analyzer (Alpkem 300 Series Autoanalyzer, ortho-PO4 manifold, EPA method 365.1, APHA (1999)). Nitrogen and phosphorus were expressed as a percentage of leaf litter AFDM, and also as the percent remaining of the total amount of each nutrient initially found in a 10-gram leaf pack. Metal content of extracts was analyzed by atomic absorption spectroscopy (AAS, Perkin Elmer AAnalyst 200) and inductively-coupled plasma mass spectroscopy (ICP-MS, Perkin Elmer Elan 6000). Extracts were amended with lanthanum chloride (LaCl3, 0.01% total volume) when analyzing for aluminum content with AAS. On days 36, 173, and 431 one replicate extract from each litter species was also analyzed for Ca, Mg, and potassium (K) content using ICP-MS.

Fungal biomass was estimated from ergosterol concentrations in preserved leaf litter samples. Ergosterol was extracted in alcoholic KOH (0.8% KOH in methanol, total extraction volume 10 ml) for 30 minutes at 80ºC in tightly capped tubes with constant stirring. The resultant crude extract was partially cleaned by solid phase extraction (Gessner and Schmitt 1996), and ergosterol quantified by high-pressure liquid chromatography (HPLC). A LichroSpher 100 RP-18 column (0.46 × 25 cm, Merck) maintained at 40°C in a Shimadzu column oven (CTO-10AS) and connected to a Shimadzu autosampler (SIL-10AD) and Shimadzu liquid chromatograph system (Pumps LC-10AT, Controller SCL-10A) was used for separation and analysis. The mobile phase was HPLC grade methanol at a flow rate of 1.5 ml min–1. Ergosterol was detected at 282 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time = ca. 8 min), and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical).

Bacterial biomass was estimated using epifluorescence direct count microscopy and analysis of captured microscope images. Bacteria attached to preserved leaf litter samples were removed by ultrasonication for 1.5 minutes using a Bransonic 150 probe sonicator, with sample tubes on ice to prevent excessive heating of the sample (Buesing and Gessner 2002). Samples were subsequently centrifuged at 800g for 1 minute in order to eliminate large suspended particles and facilitate image analysis. The supernatant from each tube was transferred to a new sterile 15-ml polystyrene tube and vortexed for 15 s to ensure homogenization of the sample. From each sample, 1 ml of formalin-preserved bacteria was filtered through a 0.2-µm Anodisc filter and stained with SYBR Gold (Patel et al. 2007). Twenty images were randomly captured from each filter at 1000X magnification using an Olympus BH-2 microscope and an Olympus Qcolor 3 digital camera (Olympus ®, Melville, NY), and analyzed using the MatLab (v 7.9) image processing toolbox. Cell counts were used to measure bacterial concentration. Biovolume

estimates (μm^3) were calculated from length (l) and width (w) measurements and converted to bacterial biomass following published protocols (First and Hollibaugh 2008).

Statistical analysis – The effects of leaf litter species and incubation length (days) on microbial respiration, fungal biomass and bacterial biomass were analyzed with multivariate analysis of covariance (MANCOVA), using PROC GLM at $\alpha = 0.05$ in SAS version 9.1 (SAS Institute Inc., Cary, USA). Time (days) was used as a covariate, leaf litter species as a fixed effect, and longitudinal location in the stream channel served as a blocking factor. If the assumption of equal covariate slopes was violated, the analysis was split three ways: one MANCOVA during the wet season before the dry period, one MANCOVA for the wet season following, and a multivariate analysis of variance (MANOVA) for the single sampling date during the dry period. MANCOVA was also used to examine the effects of leaf litter species and incubation time (days) on nutrient (N, P) and metal (Al, Fe, Mn) content in leaf litter, as well as the effects of leaf litter species on breakdown rates (k). Planned pairwise comparisons (Bonferroni method, $\alpha = 0.05$, Milliken and Johnson 1992) among leaf litter species were conducted when main effects were significant. Data were transformed whenever necessary to meet the assumptions of normality and homoskedasticity.

To determine the factors explaining nutrient immobilization and microbial respiration (O_2 uptake) in leaf litter, we compared candidate multiple regression models using Akaike's Information Criterion (AIC) and the information theoretic approach (Burnham and Anderson 2002) to select the most plausible models based on lowest AIC_c (AIC corrected for small sample size, selected based on lowest Mallows' C_p) using PROC REG at $\alpha = 0.05$ in SAS version 9.1 (SAS Institute Inc., Cary, USA). Differences between a candidate model's AIC_c and that of the top model (Δ_i), as well as Akaike weights (w_i), were calculated for all candidate models with Δ_i

not greater than ten. When summed parameter importance weights were determined, calculations were limited to a confidence set that included all candidate models with a w_i of at least 5% of the top model's w_i. For regression models dealing with respiration, samples of microbial biomass and measurements of microbial respiration were treated as subsamples and averaged per litter species on each sampling date. This was deemed necessary because samples of microbial biomass and respiration were taken from separate regions of leaf litter, and distributions of microorganisms on leaf litter are patchy (Shearer and Lane 1983). For each nutrient (N or P), the analysis was conducted for the full dataset and also separately for sampling dates during the first wet period (days 6, 36, and 62). Leaf litter species (maple, tupelo and oak) was coded as two binary variables (dummy variables "oak" and "tupelo" = 0 or 1), with a value of one for either variable signifying species identity, and zeroes for both variables indicating that the species was maple. If MANCOVA results failed to show a significant difference between the two labile litter species (maple and tupelo), leaf litter species was coded as oak (1) or not oak (0). Any explanatory variable not linearly correlated with the response variable was excluded from candidate models. Explanatory variables were tested for multicollinearity by examining Pearson's correlation coefficient matrices and calculating variance inflation factors (VIF). Variables with high VIF (> 5) and cross-correlation with other variables ($r > 0.60$) were removed from models or combined with correlated variables (Table 2.3). The average VIF in final models did not exceed the total number of explanatory variables (Neter et al. 2004), nor did it exceed 3 in any single parameter. To correct for multicollinearity in nutrient immobilization models, Al, Fe and Mn were combined into a single summed parameter (Al+Fe+Mn), and bacterial biomass (positively correlated with both metal content and fungal biomass) was excluded from models.

A model of biotic nutrient pools within leaf litter was generated using a Monte Carlo method of random sampling, conducted in R software (R Development Core Team, 2008). Empirical data on leaf litter organic matter remaining, acid-detergent fiber (ADF, cellulose + lignin), bacterial carbon, and ergosterol content collected in this study were randomly sampled with replacement, and converted to nutrient values using randomly-sampled numbers from distributions of literature values for litter nutrient leaching rates, microbial stoichiometric C:N and C:P ratios, and fungal ergosterol:C ratios (Appendix B). For detrital P, estimated biotic nutrient pools were leaf, fungal, and bacterial tissue. For detrital N, pools were the same, but the leaf tissue nutrient pool included both N complexed with ADF (ADF-N), as well as N contained in labile leaf tissue fractions (non-ADF-N). ADF-N was estimated by assuming ADF N content between 0.6-1.05% of ADF (Suberkropp et al. 1976). Resulting data were used to estimate the combined pool of nutrients in leaf tissue and bacterial and fungal biomass over time. These estimates were compared to direct measurements of total N and P in leaf litter, to determine whether nutrient content could be explained by a combination of living fungal and bacterial biomass and dead leaf tissue. The probability that estimated nutrient content was less than actual nutrient content was calculated by comparing differences in 10,000 randomly-paired estimated and observed values. Refer to Appendix B for sample calculations. Sample R code is also available upon request.

Results

Nutrient and metal content – N and P content differed among litter species (Wilks' λ = 0.17, $F_{2,57}$ = 50.33 and 62.41, respectively, all $p < 0.0001$) and increased over time (Wilks' λ = 0.11, $F_{1,57}$ = 76.75 and 177.98, respectively, all $p < 0.0001$) (Figs. 2.2A, 2.2B). All three leaf

litter species differed significantly in N content ($p \leq 0.0007$, Bonferroni) with tupelo litter containing the most and oak the least. Maple and tupelo litter had significantly higher P content than oak litter ($p < 0.0001$), but were not significantly different from one another ($p = 0.35$). Al, Fe and Mn content all differed among leaf litter species (MANOVA, Wilks' $\lambda = 0.17$, $F_{2,57} =$ 40.13, 9.25 and 9.27, respectively, all $p < 0.001$) and increased over time (Wilks' $\lambda = 0.11$, $F_{1,57} =$ 122.21, 124.00 and 170.26, respectively, all $p < 0.0001$) (Figs. 2.2C-2.2E). Maple and tupelo leaf litter displayed significantly higher Al and Fe content than oak litter (all $p < 0.006$, Bonferroni). However, tupelo had only marginally greater Al content than maple ($p = 0.0581$), and both contained similar Fe content ($p = 1.00$). There were no significant interactions between leaf litter species (treatment) and incubation time (covariate) indicating that influence of time on metal and nutrient accumulation was the same among litter species. Nutrient and metal content of leaf litter increased rapidly during the transition between wet and dry phases of the stream. Al, Fe, and P content in maple litter were second highest during the dry period, and oak litter did not begin to increase in P or Mn content until the dry period.

Changes in leaf litter N and P content over the study period were best explained by the combined effects of differences in fungal biomass and metal content (Al+Fe+Mn), with some weight of evidence ($w_i = 0.20$) for models excluding fungal biomass in the explanation of P content (Table 2.4, "N" and "P" candidate models), but none that excluded metal content. During the first wet season, roughly equivalent weight of evidence was found for fungal biomass and metal content as important parameters explaining N and P content in leaf litter (Table 2.4, "N year 1" and "P year 1" candidate models). The top candidate models explained >90% of the variation in N and P content for the full dataset, as well as during the first wet season. Bacterial

biomass was excluded from candidate regression models due to multicollinearity with Al, Fe, Mn and fungal biomass, but it was also strongly correlated with both N and P (Table 2.3).

Breakdown rate and nutrient retention (*k*) – Breakdown rates differed among tree species ($F_{2,8} = 40.48$, $p < 0.0001$, Fig. 2.3, Table 2.2), with tupelo litter exhibiting significantly greater rates than maple and oak ($p < 0.0006$, Bonferroni), which only differed from each other marginally ($p = 0.0891$). Leaf litter N and P stocks (mg pack^{-1}) differed significantly by species and time (species*days, Wilks' $\lambda = 0.11$, $F_{24,82} = 6.94$, $p < 0.0001$). Tupelo litter retained more N than oak litter after 36 days of incubation ($p < 0.05$, Bonferroni), and briefly immobilized N (percent initial N remaining > 100%), but all three leaf litter species showed a net loss of N (percent initial remaining < 100%) by the end of the first wet season (Fig. 2.4, observed). During the dry period, litter species differed in percent initial N remaining, with maple and oak litter both immobilizing N, and retaining significantly greater N stocks when compared to tupelo litter (all $p < 0.01$, Bonferroni). During the second wet season, oak litter retained a greater stock of N than it contained prior to incubation or during the first wet season. In addition, both oak and maple litter retained more N than tupelo litter (all $p < 0.01$, Bonferroni), primarily due to the rapid breakdown of tupelo litter.

Initial stocks of P (prior to incubation) were significantly greater in maple and tupelo litter than in oak litter (all $p < 0.05$, Bonferroni). Leaf litter acted as a net source of P during the first wet season, with decreases in percent P remaining over time, but differences among litter species were only marginal. Oak litter experienced greater relative decreases than maple or tupelo litter (all $p < 0.05$, Bonferroni), with 52 ± 3 percent of its initial mass of P remaining at the end of the first wet season (62 days), compared to 72 ± 2 and 60 ± 2 percent remaining in maple and tupelo litter, respectively. During the dry season, maple litter contained significantly

more P than did oak or tupelo litter (all $p < 0.005$, Bonferroni). Tupelo litter was lowest in percentage of its initial P stock during the dry period (54 ± 14 %, $p = 0.022$, Bonferroni), while oak and maple litter retained 97 ± 9 and 127 ± 12 percent of their initial P stock, respectively. During the second wet season, tupelo litter contained significantly less P than its initial stock (all $p < 0.001$, Bonferroni), but maple and oak litter's current stocks did not differ from those prior to incubation. Oak litter retained a total of 2.57 ± 0.09 (1 S.E.) mg P after 431 days of breakdown, which was a greater quantity than initially present in 10 g of oak litter (2.31 ± 0.83 mg P), and greater than the amount remaining in labile tupelo litter (1.29 ± 0.24 mg P) after 431 days of breakdown. However, although maple was a net source of P during the second wet season (percent initial P remaining < 100), it retained the greatest absolute amount of P (3.10 ± 0.44 mg P) at the end of the study.

Potential biotic contributions to detrital N and P: Modeling results – Modeling results suggest that estimated biotic pools of N cannot fully account for the entire mass of nutrients measured directly, especially after longer incubations (Fig. 2.4A-C). This differs among leaf litter species, with a greater probability (33 ± 19% [± 1 95% C.I.]) that oak litter N can be explained by biotic drivers across incubation times. Estimated labile and recalcitrant fractions of leaf tissue held the majority of observed N initially (82% in tupelo -98% in maple), and remained the dominant pool even after long incubations (46% in maple - 50% in oak). Median potential bacterial contributions to observed detrital N were low and averaged 0.4% (range 0.05% in oak litter-1.34% in tupelo litter) across incubation times and litter species, assuming a redfield C:N ratio (6.625) resulted in lower potential bacterial contributions (0.04-1.13%) to detrital N. Potential contributions by living fungal biomass to observed detrital N ranged from 4-19%, 7-16%, and 8-11% in oak, tupelo, and maple litter, respectively, and were highest in oak litter

during the dry period (19%). Fixed ergosterol:fungal dry mass (5.5) and Redfield C:N (6.625) ratios provided higher estimates of fungal contributions to detrital N (5-27%), primarily because the Redfield C:N ratio is at the low end of values measured directly (6.083-13.75, Newell and Statzell-Tallman, 1982, and 7-16, Leach and Gulis 2011, personal communication).

Estimated biotic nutrient pools had a higher probability of accounting for total detrital P (Fig. 2.5 A-C) than detrital N, although the probability decreased after longer incubations, as was the case for N. Biotic contributions to total P also had a higher probability in oak litter – compared to other litter types – of accounting for observed detrital P across incubation times (57 ± 13% [± 1 95% C.I.]). Unlike estimates of detrital N, which were dominated by nutrients contained in leaf tissue, microbial P accounted for the largest estimated relative contribution to observed P in almost half (7/15) of all estimates. The probability of accounting for observed detrital P when allowing for flexible fungal and bacterial C:P ratios was highest in 13/15 of all estimates (Fig. 5 A-C), as direct measurements of fungal (40-203, Leach and Gulis 2011, personal communication) and bacterial (8-260) C:P ratios allow for higher P content than the Redfield ratio (106). Median bacterial P contributions to observed detrital P were small (average 1%, range 0.25% in oak – 5% in tupelo), although higher than bacterial contributions to observed N. A Redfield C:P ratio (106) resulted in lower potential bacterial contributions (0.13-3%) to detrital P. Estimated fungal P accounted for the largest relative proportion (36 ± 8% [± 1 95% C.I.]) of observed P. Median potential contributions by living fungal biomass to observed detrital P ranged from 16 -73%, 21-59%, and 21-34% in oak, tupelo, and maple litter, and were highest in oak litter during the dry period (73%). The discrepancy between estimated and observed detrital P stocks (Fig. 2.5D) was positively correlated to mg of Al ($t_{1,13} = 4.56$, $p < 0.001$, $r^2_{adj.} =$

0.59, Fig. 2.5D) Fe ($t_{1,13} = 3.81$, $p < 0.005$, $r^2_{adj.} = 0.49$), and bulk inorganic matter ($t_{1,13} = 4.27$, $p < 0.001$, $r^2_{adj.} = 0.55$) per litter pack.

Microbial biomass and respiration – Microbial respiration (Fig. 2.6) and fungal and bacterial biomass (Fig. 2.2G, Fig. 2.2H) differed among litter species and changed over time, but fungal biomass and microbial respiration became more similar among litter species during the second wet season. Significant interactions between leaf litter species (treatment) and incubation time (covariate) indicates that in contrast with metal and nutrient content, incubation time influenced fungal and bacterial biomass and microbial respiration differently across litter species. Therefore, we analyzed the two wet seasons and the single dry season separately (see methods). During the first wet season, microbial respiration rates differed among litter species (Wilks' $\lambda = 0.12$, $F_{2,39} = 5.24$, $p < 0.0097$, Fig. 2.6) and increased over time (Wilks' $\lambda = 0.51$, $F_{1,39} = 12.27$, $p = 0.0012$, Fig. 2.6), with tupelo litter exhibiting significantly higher microbial respiration rates than oak litter ($p = 0.0071$, Bonferroni). Microbial biomass (below) and respiration were not measured in tupelo litter during the second wet season due to the limited amounts of litter remaining.

Fungal biomass differed among leaf litter species ($F_{2,39} = 33.25$, $p < 0.0001$) and increased over time in maple and oak litter ($F_{1,39} = 16.19$, $p = 0.0003$, Fig. 2.2G), with the highest biomass observed on tupelo litter, followed by maple and then oak litter ($p < 0.002$, Bonferroni). Bacterial biomass also differed among litter species (Wilks' $\lambda = 0.12$, species $F_{2,39} = 33.38$, $p < 0.0001$) and increased over time (Wilks' $\lambda = 0.51$, $F_{1,39} = 18.09$, $p = 0.0001$, Fig. 2.2H) during the first wet season, with the highest biomass also observed on tupelo litter, followed by maple and then oak ($p < 0.008$, Bonferroni).

During the second wet season, differences in microbial respiration rates between maple and oak litter were only marginally significant (Wilks' $\lambda = 0.16$, $F_{1,13} = 4.52$, $p = 0.0532$, Fig. 2.6), and interspecies differences in fungal biomass also became less pronounced (Wilks' $\lambda = 0.16$, $F_{1, 13} = 1.78$, $p = 0.21$, Fig. 2.2G). Contrary to our predictions, bacterial biomass on maple litter remained higher than corresponding estimates on oak litter (Wilks' $\lambda = 0.16$, $F_{1,13} = 58.50$, $p < 0.001$, Fig. 2.2H), with greater differences between the two species than were observed during the first wet season. Overall differences in microbial respiration rates among litter species were best explained by fungal and bacterial biomass and ambient temperature, with 1.75× higher weight of evidence for fungal biomass than bacterial biomass as an important parameter influencing total microbial respiration (Figure 2.6, Table 2.5).

Discussion

The degree to which leaf litter acts as a sink for nutrients over time is determined by the tree species from which it was derived, with litter species traits modifying a complex set of biotic and abiotic processes occurring in the detrital matrix during decomposition. Here we provide evidence that metals may an important component of nutrient accumulation in detritus. Nutrient uptake and retention in leaf litter is facilitated by microbial growth and activity, but is also influenced by the degree to which litter intercepts particles from the surrounding water column or facilitates the *in situ* precipitation of particles from adsorbed metals. The activity of consumers and decomposers may further modify these dynamics by altering rates of breakdown and decomposition; while labile leaf litter initially supports higher microbial biomass, it is rapidly lost from the system, making recalcitrant leaf litter a potentially larger long-term sink for nutrients.

Modeling results show that a large portion of the detrital nutrients are not accounted for by biotic components, even when allowing for a great deal of uncertainty in litter nutrient leaching rates, microbial C:nutrient conversion factors, and microbial stoichiometric ratios. The ability of leaf litter to immobilize nutrients over time is determined by the tree species from which it was derived, with species traits (i.e., plant litter chemistry) modifying a complex set of biotic and abiotic processes occurring in the detrital matrix during litter breakdown. A better understanding of microbial stoichiometry and its changes throughout the breakdown process would aid in our understanding of the drivers regulating detrital nutrient content. Furthermore, nutrient uptake and retention in leaf litter is facilitated by microbial growth and activity, but may also be influenced by interception of sediment particles (i.e., metals) from the surrounding water column by litter.

Microbial stoichiometry and its influence on detrital nutrient content – Estimates of microbial nutrient content based solely on measured cellular components (i.e. chitin, ATP, or ergosterol) involve a great deal of uncertainty. Ergosterol is a reliable estimate, but not exact measurement of fungal biomass, since ergosterol:dry mass ratios are known to vary considerably among species and also within a species depending on age, oxygen, and nutrient availability (Gessner and Chauvet 1993, Charcosset and Chauvet 2001). The upper limits of the confidence intervals in figures 4 and 5 illustrate the extremes that are possible if allowing for a combination of high microbial nutrient content, low ergosterol:dry mass ratios in fungi, low leaching rates of leaf nutrients, and high concentrations of N contained in recalcitrant leaf tissue. Therefore, while it is theoretically possible to account for the entire mass of nutrients contained in leaf litter with biotic drivers, it is not highly probable.

The high fungal nutrient contribution to oak leaf nutrient content during the dry period were surprising, given the recalcitrant nature of the leaves, presumably lower moisture in leaf tissues during the dry period, and lower fungal biomass in oak litter during other times of the year. However, fungal N and P contributions to detrital nutrients greater than 50% have been observed in other systems (Kuehn et al. 2011). High fungal biomass (highest for oak litter) during the dry period may be due to the exploitation of high concentrations of lignin and lignin-bound N in oak leaf tissue, the breakdown of which requires oxygen (Gubernatorova and Dolgonosov 2010).

Missing nutrients – Several biotic and abiotic pools may contribute to the observed detrital N and P not accounted for by our models. In the case of N, we were unable (even the upper limits of our estimates) to account for all litter nutrients using the measured biotic pools, suggesting that additional detrital N was present in either biotic or abiotic pools that had not been measured. Findlay et al. (2002) found in a cross-system comparison that fungal biomass (measured as ergosterol) was a poor predictor of detrital C:N ratios. Immediately after leaf litter enters the stream and immediately following dry periods (6 and 346 days), some terrestrial microbial N and P may remain in litter. Oomycetes, which include fungal-like mycelial decomposers not possessing ergosterol, are early colonizers of leaf litter in streams that can exibit rapid growth (Nikolcheva and Bärlocher 2004, Mille-lindblom et al. 2006) and may account for some N and P not accounted for by fungal biomass estimated from ergosterol measurements. As Oomycete biomass tends to decline rapidly (Bärlocher 1990), their effects on detrital stoichiometry may be diminished in later stages of leaf decay. Furthermore, the microbial community undergoes successional changes over time (Gessner et al. 1993), which could alter the overall stoichometry of the microbial community. Although our estimated median values of

microbial contributions to detrital nutrients are within the range of theoretically possible values, pinpointing the exact median value of microbial nutrients would require a better understanding of the microbial community structure and their underlying nutrient stoichometry.

A large proportion of N may be contained in residual fungal cellular components – such as chitin – which were not measured here. Chitin is a primary component of fungal cell walls and contains up to 7% nitrogen, although it breaks down very slowly (Swift et al. 1979, Ekblad et al. 1998). Stream drying and rewetting may cause major changes in microbial biomass (Bruder et al. 2011) and a potential restructuring of microbial communities between terrestrial-species-dominated and aquatic-species-dominated composition. If this were the case, a large amount of chitin in residual terrestrial fungal tissue could explain our inability to account for observed detrital N following the dry period. This potentially significant fungal N contribution would be underestimated based on ergosterol assays alone.

N bound to lignin and cellulose fractions of litter is another recalcitrant pool of nutrients rarely measured directly (Fioretto et al. 2005). Prior to leaf abscission, leaf proteins form complexes with phenolic compounds that may combine with lignin and cellulose to form recalcitrant pools of N (lignoproteins), which can make up a significant proportion of total N in leaf tissue (Brinkmann et al. 2002). Suberkropp et al. (1976) proposed that N-containing microbial exoenzymes may form additional complexes with lignoproteins throughout the breakdown process, resulting in an increasing concentration of N associated with recalcitrant structural leaf compounds over time. Schlesinger and Hasey (1981) speculated that while microbial exoenzymes may be responsible, apparent N increases in ADF-fractions of litter may also include chitin, which is resistant to H_2SO_4-extraction methods commonly used in gravimetric determination of lignin. However, there are no known pools of recalcitrant P in

microbial or plant tissues. Because of significant correlations between concentrations of P and accumulated metals, and correlations between "missing" P and accumulated metals, it seems probable that P bound to clays and other inorganic sediments may make up a portion of the nutrients yet unaccounted for.

Influence of leaf structure on nutrient and metal dynamics in detritus

Leaf litter chemistry influences initial colonization and growth of N- and P-sequestering microorganisms, and may also influence the initial development of particle-trapping biofilms, but physical structures on leaf surfaces may have played a role as well. Litter species in the current study differed greatly in the density of hairs (pubescence) on their surfaces. Pubescence would likely facilitate the initial attachment stage of bacterial biofilm development and may also increase the accumulation of suspended particles from the stream (Dang et al. 2007). The most pubescent of the three litter species (tupelo) had the greatest total amount of inorganic matter (including metals and nutrients) per gram and per unit area of leaf surface throughout the study. The importance of species identity as a model parameter accounts for differences in initial nutrient content among litter species, but also suggests that species traits that are more difficult to quantify, such as surface roughness, may also contribute to nutrient dynamics.

Litter recalcitrance also has the potential to have long-lasting ecosystem effects on nutrient retention, by way of sheer resilience. Litter breakdown rates in this study were slowest for litter with highest ratios of structural compounds (i.e. lignin) to nutrients, as has been demonstrated in previous research (Melillo et al. 1982). Although oak had lower concentrations of nutrients and metals than other litter species, it decayed slowly enough that toward the later stages of breakdown it became a net sink for N and P. At different points in time all three species became sinks for N (% initial remaining greater than 100%), and maple and oak also became net

sinks for P, but this was delayed for more recalcitrant species and occurred earliest in labile litter species. Therefore, recalcitrant leaf litter may slow nutrient and metal export to downstream reaches more effectively than labile litter over time.

Accumulated metals as a nutrient storage pool – Our findings are consistent with research highlighting a strong influence of microbial growth on detrital nutrient content (Gulis et al. 2006, Kuehn et al. 2011) but suggest that in addition to microbial community structure and nutrient stoichiometry, a better understanding of detrital accumulation of metals may aid in our understanding of detrital nutrient dynamics (Hall et al. 2011). Strong correlation between bacterial biomass and Al, Fe and Mn content suggests bacterial biofilms may be central to the process of particle interception and metal accumulation. As bacterial biofilms develop on submerged litter surfaces they may enhance adsorption of metals (Ferris et al. 1989) and other particles (Battin et al. 2003). Thus, the potential for metal adsorption as an additional driver of nutrient uptake should be viewed as a coupled biotic-abiotic process. Microbial activity in leaf litter biofilms can influence the rate of metal-oxide accumulation (Ferris et al. 1999) and thereby indirectly enhance nutrient immobilization.

Iron, manganese, and aluminum content were strongly correlated in this study, and all three metals may have been accumulating in the detrital matrix as co-precipitates in metal oxides or as coatings on larger particles. However, the fact that Al, Fe, Mn and Si content increased over time, while Ca, Mg, and K content decreased (Appendix A), indicates that the relative concentrations of elements in leaf-litter-associated inorganic matter were influenced by more than passive accumulation of sediment. Al and Si content were lower than those measured in regional soils, however, Fe content was higher than that of coastal plain soils suggesting selective accumulation within the litter-biofilm matrix.

In aquatic environments biofilms have been shown to accumulate cations such as Al, Ca, Fe, Mg and Mn up to 21,000× above stream water concentrations (Lalonde et al. 2007), and to precipitate clay-like (Fe,Al)-silicates (Konhauser and Urrutia 1999). Association of these metals with clay particles also explains the correlation between nitrogen and metals in leaf litter, as ammonium and dissolved organic nitrogen, the two most abundant forms of N in stream water from the study site, are strongly adsorbed to clay (Triska et al. 1994, Aufdenkampe et al. 2001). Consistent with this conceptual framework, respiration rates were strongly affected by temperature and were also significantly correlated to fungal and bacterial biomass, suggesting an active microbial community. Oak litter, which had the least metabolically active microbial community throughout the study, also immobilized significantly less nutrients and metals per gram of litter.

Nutrients adsorbed to metal oxides and other inorganic matter may be less bioavailable to microorganisms and higher trophic levels, depending on the elements involved. Production of Al- and Fe-solubilizing acids has been documented in fungi and bacteria (Gensemer and Playle 1999, Das et al. 2007), and iron reduction by bacteria in leaf litter biofilms may gradually liberate Fe-bound phosphorus as well (Burgin et al. 2011). It is possible that adsorbed N and P could still be assimilated in the gut of consumers, depending on the element that nutrients are bound. Al only becomes soluble at pH levels lower than those observed in the guts of most aquatic macroinvertebrates, and it is relatively unaffected by changes in redox conditions. However, iron reduction has been demonstrated in terrestrial insects (Vu et al. 2004). The extremely low redox potential in the anoxic guts of many aquatic macroinvertebrates (Stief et al. 2009) makes liberation of phosphorus during digestion via an Fe-reduction mechanism possible. This may represent an additional pathway for leaf litter nutrient flow into higher trophic levels of

aquatic food webs, without directly obtaining nutrients from ingested microorganisms or plant tissue, but the degree to which this occurs is unknown.

Summary – Detrital nutrient content is commonly expressed relative to the dry weight of the organic fraction of litter, although much of the nutrients could be incorporated in—and partially a function of—the inorganic fraction of the detrital matrix. The dynamics of this potentially significant component of detritus are rarely examined in aquatic studies, but may be essential to a more complete understanding of detrital nutrient dynamics. Furthermore, because accumulation rates of metals and retention of nutrients differ significantly among litter species, our findings suggest that forest composition may be able to influence nutrient and metal cycling across regional scales in rivers.

Acknowledgements

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Table 2.1: Physical and chemical data (mean ± 1 S.E.) within the Little River Experimental Watershed averaged across sampling dates. For DOC (mg L^{-1}), N (µg L^{-1}), and P (µg L^{-1}), n = 31; for DO (mg L^{-1}), pH, and temperature (°C), n = 10; and for Fe and Mn (both in µg L^{-1}), n = 7.

DO	7.51	±	0.72
DOC	10.76	±	0.50
pH	6.82	±	0.28
temperature	13.91	±	1.15
Total P	28.92	±	6.77
PO_4^{3-}	7.21	±	2.21
Total N	562.38	±	139.90
NO_3^-	9.29	±	2.64
NH_4^+	26.88	±	3.69
Fe	65.71	±	15.54
Mn	31.49	±	5.57

Table 2.2: Mean breakdown rates and initial percent concentrations of leaf litter structural compounds and nutrients. Standard errors (± 1 S.E.) are provided in parentheses next to mean values. For breakdown rates (*k*) and initial (pre-incubation) percent concentrations of leaf litter structural compounds, n = 5. For nutrient concentrations, n = 3.

Leaf litter species	*k*	lignin	hemicellulose	cellulose	C	N	P	lignin:N
Ogeechee tupelo	0.0053 (0.0006)	8.18 (0.48)	13.04 (0.40)	19.07 (0.49)	48.67 (0.02)	1.04 (0.08)	0.038 (0.006)	8.48 (0.95)
trident red maple	0.0024 (0.0002)	13.29 (0.23)	10.52 (0.52)	20.08 (0.82)	50.04 (0.19)	0.97 (0.06)	0.038 (0.002)	13.86 (0.52)
water oak	0.0013 (0.0002)	13.56 (0.51)	12.72 (0.54)	22.76 (1.06)	50.57 (0.11)	0.83 (0.11)	0.021 (0.005)	16.71 (1.46)

Table 2.3: Pearson correlation coefficients of parameters used in model comparisons.

	Fungi	**Al**	**Fe**	**Mn**	***N***	***P***
Bacteria	.61	.85	.81	.79	*.82*	*.75*
Fungi	-	.43	.45	.43	*.70*	*.61*
Al		-	.95	.88	*.81*	*.91*
Fe			-	.84	*.80*	*.89*
Mn				-	*.63*	*.76*
N					-	*.91*

Table 2.4: Comparison of candidate multiple regression models explaining variation in nitrogen (N) and phosphorus (P) content of leaf litter for full datasets (N, P) and during the first wet season only (N year 1, P year 1). *K* is the number of parameters in the multiple regression model (including y-intercept and error), C_p is Mallows' C_p, AIC_c is Akaike's second-order information criterion (corrected for small sample size), Δ_i is the difference between the candidate model and the best model's AIC_c, *L* is the likelihood value of each model, and w_i is the relative strength of evidence for each candidate model (between 0-1).

N	***K***	C_p	AIC_c	Δ_i	***L***	w_i
candidate model						
fungi, Al+Fe+Mn, oak, tupelo	6	5.00	-188.13		1	0.78
fungi, Al+Fe+Mn, oak	5	7.99	-185.58	2.55	0.28	0.22
N year 1	***K***	C_p	AIC_c	Δ_i	***L***	w_i
candidate model						
fungi, Al+Fe+Mn, oak, tupelo	6	5.00	-123.61		1	0.69
fungi, Al+Fe+Mn, oak	5	7.58	-121.85	1.76	0.41	0.29
fungi, oak, tupelo	5	14.22	-115.83	7.78	0.02	0.01
fungi, oak	4	18.37	-114.28	9.33	0.01	0.01
P	***K***	C_p	AIC_c	Δ_i	***L***	w_i
candidate model						
fungi, Al+Fe+Mn, oak	5	4.00	-785.93		1	0.80
Al+Fe+Mn, oak	4	7.17	-783.19	2.75	0.25	0.20
P year 1	***K***	C_p	AIC_c	Δ_i	***L***	w_i
candidate model						
fungi, Al+Fe+Mn, oak	5	4.00	-539.37		1	0.54
Al+Fe+Mn, oak	4	6.26	-537.82	1.55	0.46	0.25
fungi, oak	4	6.58	-537.51	1.86	0.39	0.21

Table 2.5: Comparison of candidate multiple regression models explaining variation in oxygen uptake generated by leaf litter. *K* is the number of parameters in the multiple regression model (including y-intercept and error), C_p is Mallows' C_p, AIC_c is Akaike's second-order information criterion (corrected for small sample size), Δ_i is the difference between the candidate model and the best model's AIC_c, *L* is the likelihood value of each model, and w_i is the relative strength of evidence for each candidate model (between 0-1). Parameter importance weights are calculated as the sum of the values of w_i for all models containing the parameter of interest.

candidate model	***K***	C_p	AIC_c	Δ_i	***L***	w_i
fungi, temperature	4	4.00	-31.35		1	0.46
fungi, bacteria, temperature	5	4.59	-30.83	0.52	0.77	0.36
bacteria, temperature	4	7.12	-28.80	2.55	0.28	0.13
fungi	3	11.25	-25.66	5.69	0.06	0.03
fungi, bacteria	4	12.76	-25.20	6.14	0.05	0.02
bacteria	3	17.70	-22.43	8.92	0.01	0.01

parameter	**temperature**	**fungi**	**bacteria**
importance weight	0.95	0.84	0.48

Figure 2.1: Discharge and sampling dates in the study reach of the LREW. Arrows indicate sampling dates, with incubation time in days listed above each arrow. Shaded areas indicate dry periods when flow ceased and the stream channel dried.

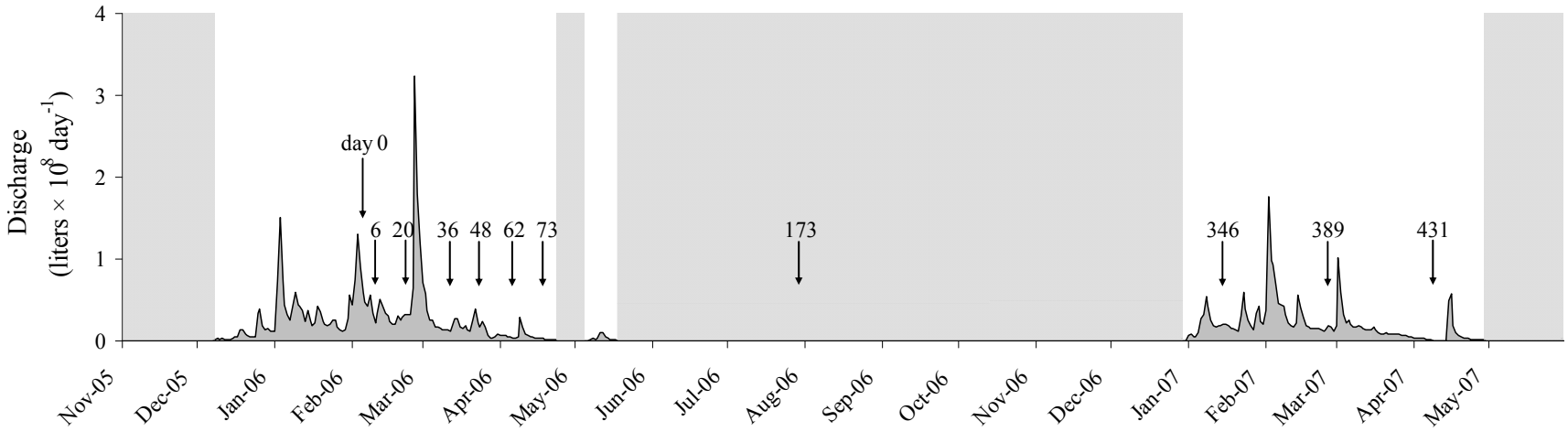
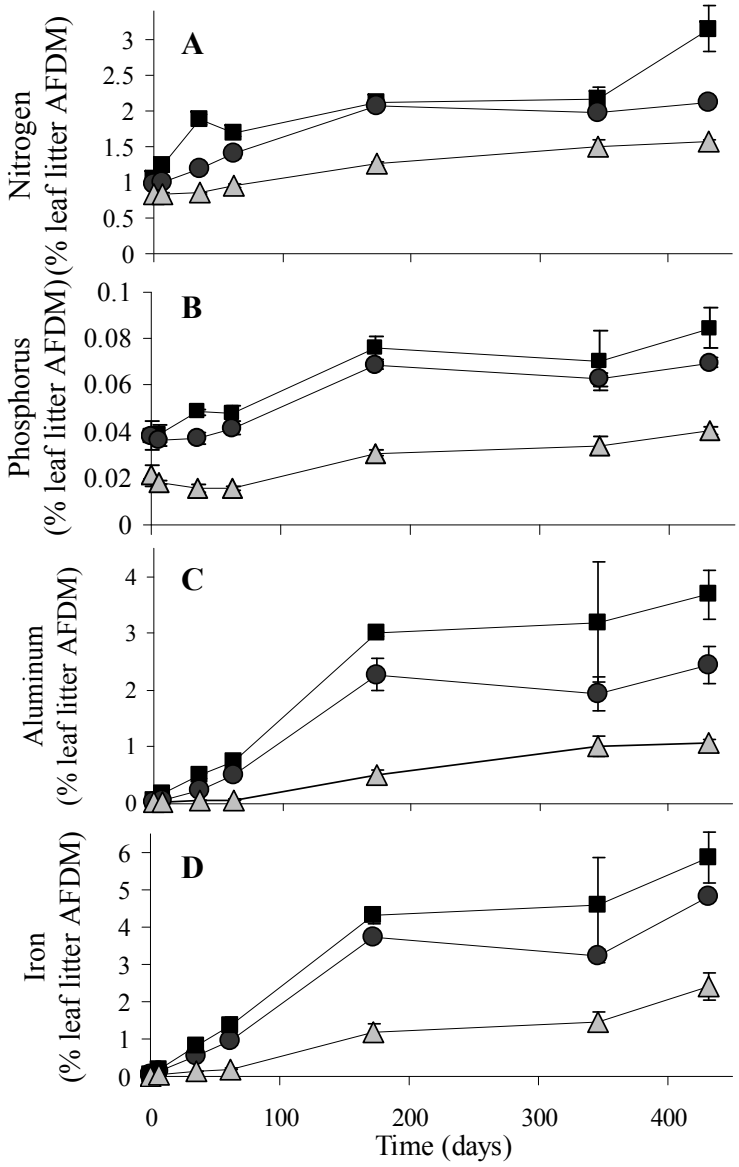

Discharge
(liters × 10^8 day^-1)
4
3
2
1
0
day 0
6
20
36
48
62
73
173
346
389
431
Nov-05
Dec-05
Jan-06
Feb-06
Mar-06
Apr-06
May-06
Jun-06
Jul-06
Aug-06
Sep-06
Oct-06
Nov-06
Dec-06
Jan-07
Feb-07
Mar-07
Apr-07
May-07

Figure 2.2: Changes in concentrations of leaf litter nutrients, metal oxides, inorganic matter, fungal biomass, and bacterial biomass over time. Mean leaf litter (A) nitrogen, (B) phosphorus, (C) aluminum, (D) iron, (E) manganese, (F) inorganic matter (n=5), (G) fungal biomass (n=5), and (H) bacterial biomass (n=5) are expressed per leaf litter AFDM. Vertical bars around means (sometimes obscured by symbols) signify ± 1 standard error. All n = 3 unless otherwise noted.


A
Nitrogen
(% leaf litter AFDM)
B
Phosphorus
(% leaf litter AFDM)
C
Aluminum
(% leaf litter AFDM)
D
Iron
(% leaf litter AFDM)
Time (days)

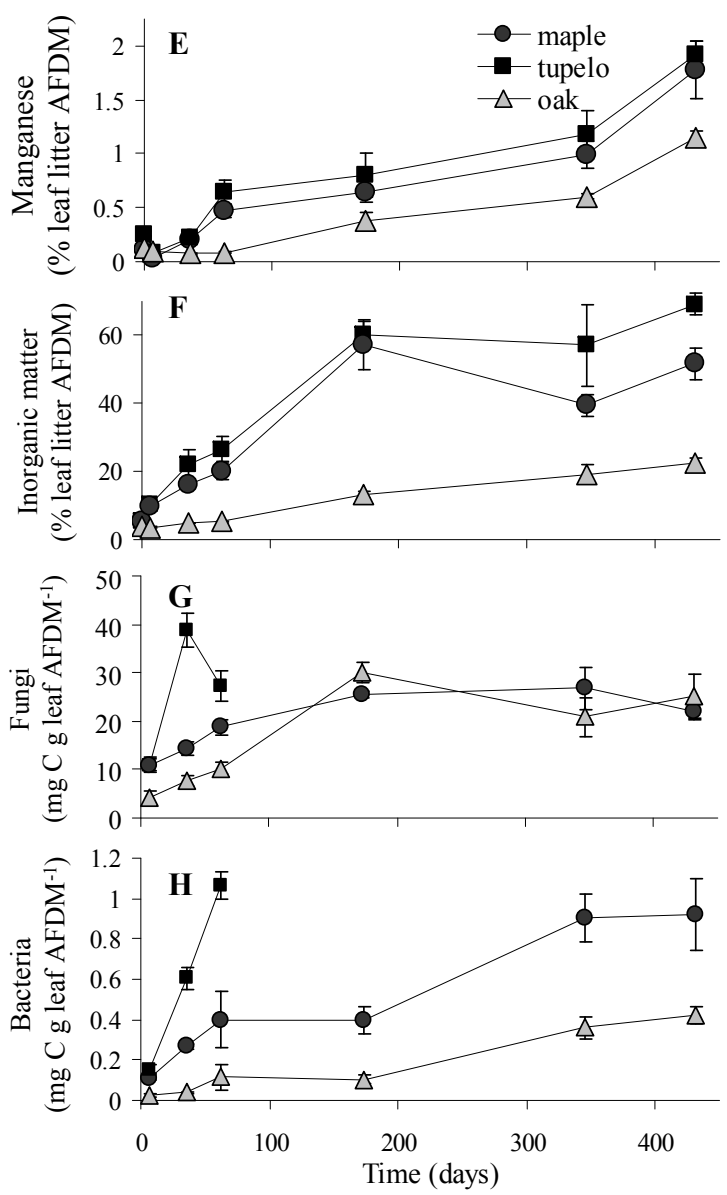

maple
tupelo
oak
E
Manganese
(% leaf litter AFDM)
F
Inorganic matter
(% leaf litter AFDM)
G
Fungi
(mg C g leaf AFDM-1)
H
Bacteria
(mg C g leaf AFDM-1)
Time (days)

Figure 2.3: Mean fractions of leaf litter organic matter (non-fibrous, cellulose, hemicellulose, lignin) remaining over time per leaf litter species. On day 173, fibrous fractions were not measured directly and were assumed equal to those on day 62.

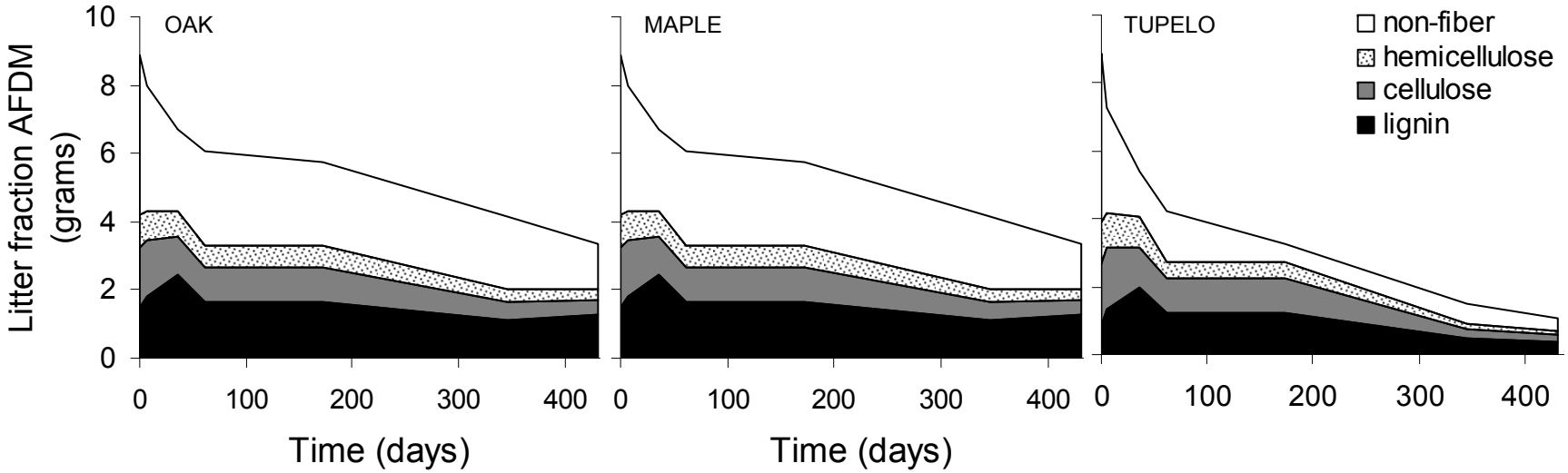

Litter fraction AFDM (grams)
10
8
6
4
2
0
OAK
MAPLE
TUPELO
non-fiber
hemicellulose
cellulose
lignin
0
100
200
300
400
Time (days)

Figure 2.4: Median observed and modeled detrital nitrogen remaining over time in A) oak, B) tupelo and C) maple leaf litter. For each incubation time (in days), columns (from left to right) are 1) N predicted with flexible ergosterol:dry mass (2.3-11.5 μg g^{-1}), fungal C:N (6.083-16), bacterial C:N (2.62-17.1) ratios, N concentration in acid-detergent fiber (ADF) and non-ADF tissues in leaf litter, and 2) N measured directly (observed). The probability that N estimated with flexible stoichiometric ratios (and fixed ratios, in parentheses) is equal to or greater than observed N is provided above each column. Error bars represent 95% quantile ranges (confidence intervals). At t=173 days, the stream channel was completely dry.

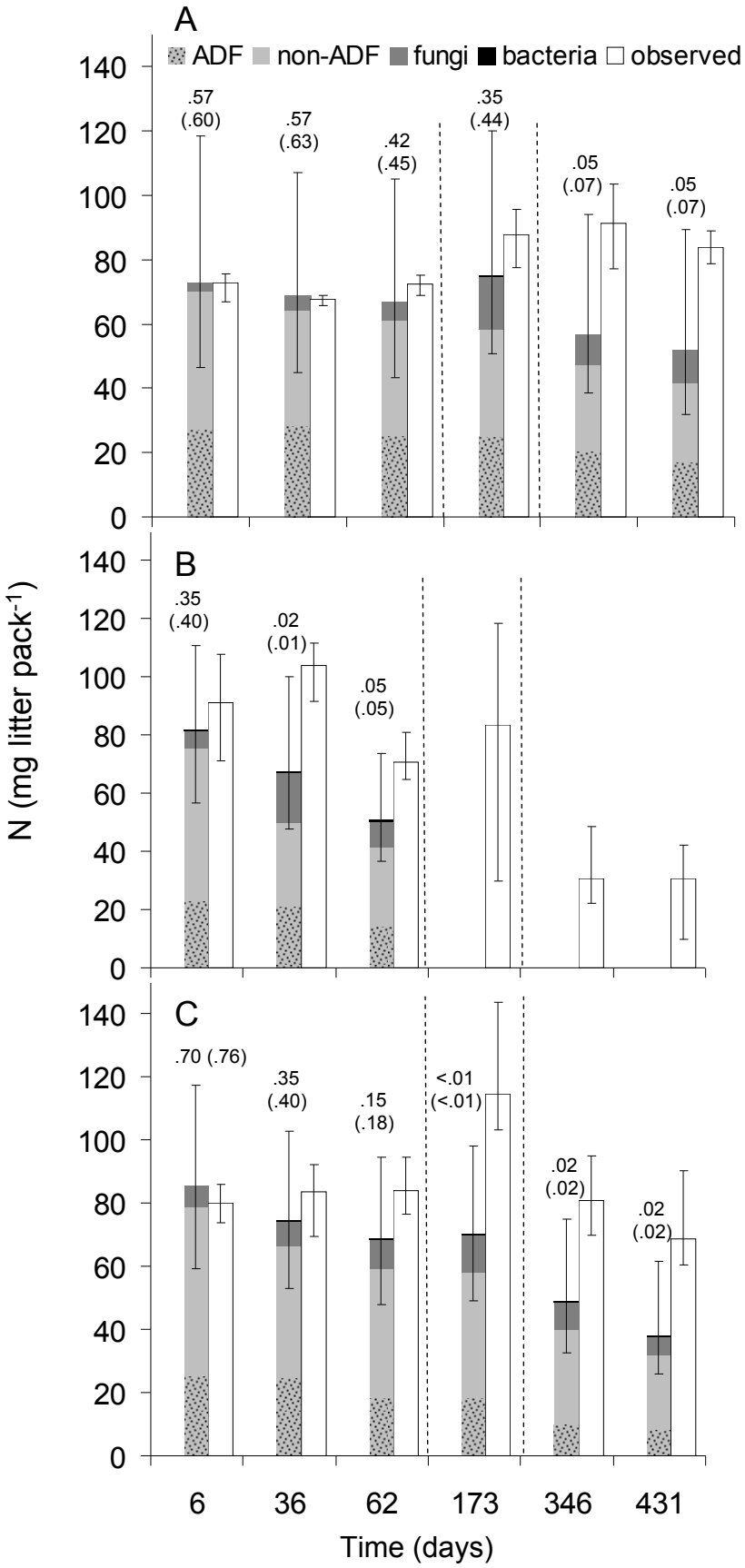

A
ADF
non-ADF
fungi
bacteria
observed
.57 (.60)
.57 (.63)
.42 (.45)
.35 (.44)
.05 (.07)
.05 (.07)
B
.35 (.40)
.02 (.01)
.05 (.05)
C
.70 (.76)
.35 (.40)
.15 (.18)
<.01 (<.01)
.02 (.02)
.02 (.02)
N (mg litter pack-1)
0
20
40
60
80
100
120
140
6
36
62
173
346
431
Time (days)

Figure 2.5: Median observed and modeled detrital phosphorus remaining over time in A) oak, B) tupelo and C) maple leaf litter. For each incubation time (in days), columns (from left to right) are 1) P predicted with flexible ergosterol:dry mass (2.3-11.5 μg g^{-1}), fungal C:P (40-203), and bacterial C:P (8-260) ratios, and 2) P measured directly (observed). The probability that P estimated with flexible stoichiometric ratios (and fixed ratios, in parentheses) is equal to or greater than observed P is provided above each column. Error bars represent 95% quantile ranges (confidence intervals). At t=173 days, the stream channel was completely dry. D) Regression of the observed (obs.) minus predicted (pred.) median values of detrital P against mass of Al in maple, tupelo, and oak litter packs (mg $pack^{-1}$).

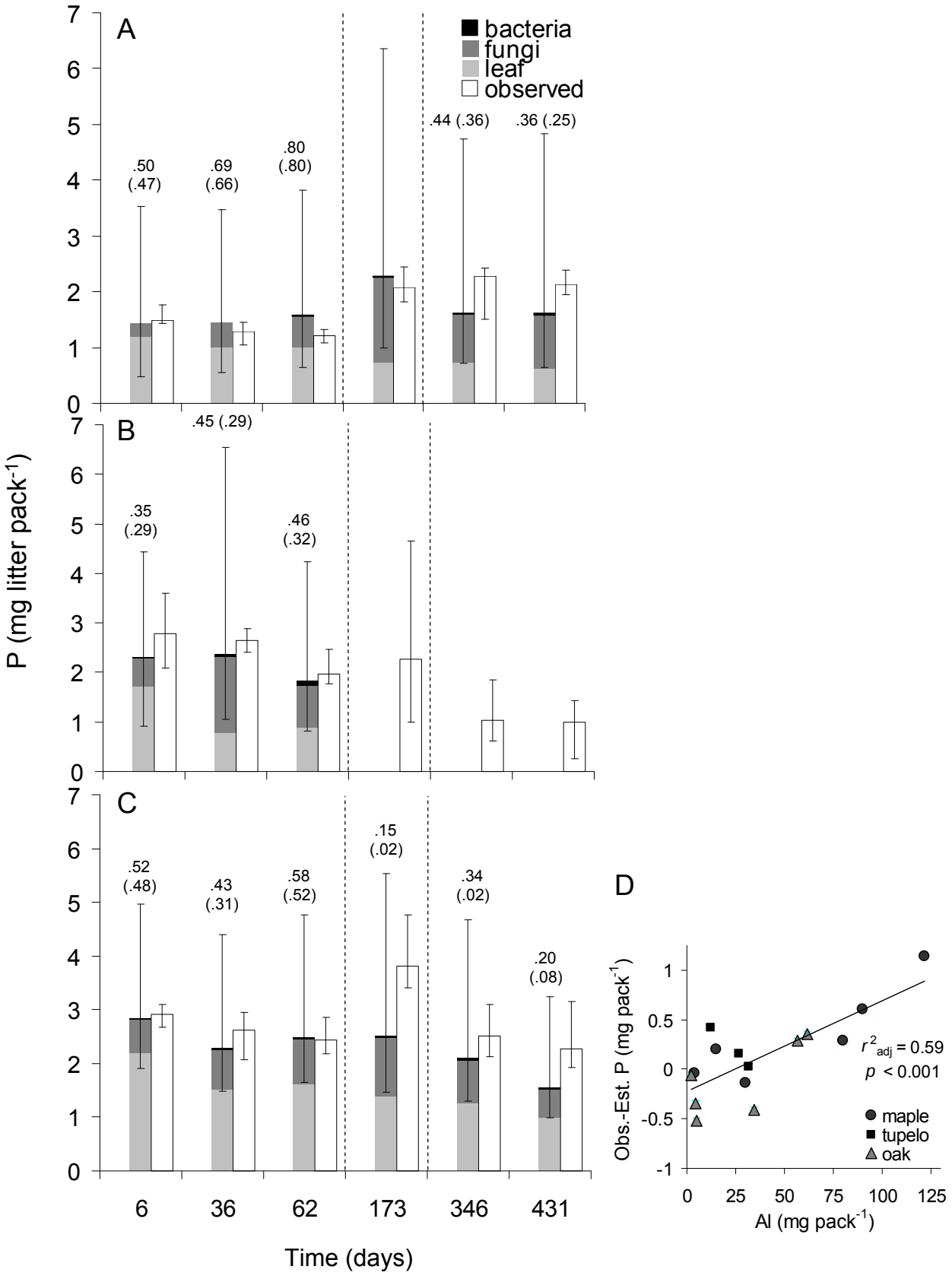
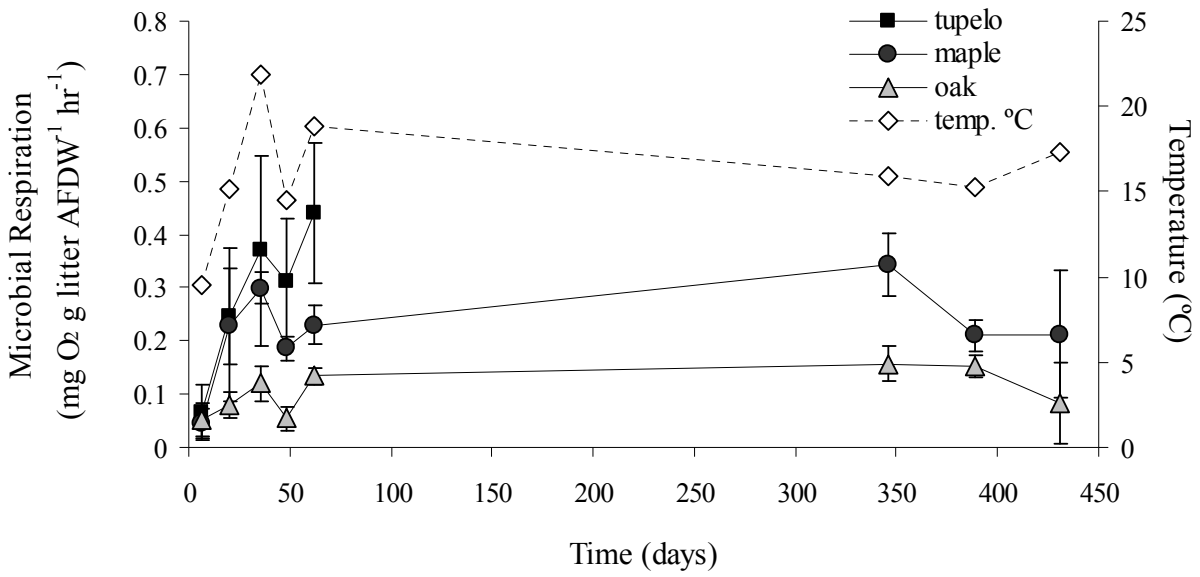

A
B
C
D
bacteria
fungi
leaf
observed
.50 (.47)
.69 (.66)
.80 (.80)
.44 (.36)
.36 (.25)
.45 (.29)
.35 (.29)
.46 (.32)
.52 (.48)
.43 (.31)
.58 (.52)
.15 (.02)
.34 (.02)
.20 (.08)
P (mg litter pack-1)
Time (days)
6
36
62
173
346
431
Obs.-Est. P (mg pack-1)
Al (mg pack-1)
r2adj = 0.59
p < 0.001
maple
tupelo
oak

Figure 2.6: Mean microbial oxygen uptake rates over time per leaf litter species. Error bars signify ± 1 standard error. For all dates and leaf litter species, n = 5.


Microbial Respiration
(mg O2 g litter AFDW-1 hr-1)
Temperature (ºC)
Time (days)
tupelo
maple
oak
temp. ºC
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
5
10
15
20
25
0
50
100
150
200
250
300
350
400
450

CHAPTER 3

LEAF LITTER-ASSOCIATED OXYGEN DEMAND IN A BLACKWATER RIVER DRAINING GEORGIA'S COASTAL PLAIN: THE EFFECTS OF LITTER CHEMISTRY, FUNGI, BACTERIA, AND MACROINVERTEBRATES[1]

[1]Andrew S. Mehring, George Vellidis, Kevin A. Kuehn, Catherine M. Pringle, M.R. First, and R. Richard Lowrance. To be submitted to *Oecologia*.

Abstract

Leaf-litter-generated oxygen demand in blackwater swamps and streams is controlled by a complex interaction between litter chemistry and numerous members of the decomposer guild. Differences in microbial biomass among leaf litter species may be affected by initial litter chemistry, but these chemical differences may change over time due to accrual of microbial biomass and degradation of labile compounds in leaf litter. Although labile litter may support initially high microbial oxygen uptake rates, it breaks down rapidly due to the activity of microbes and larger detritivores, and it may have reduced long-term contributions to oxygen demand relative to recalcitrant litter. This conceptual model has rarely been tested in intermittent blackwater streams. To this end, leaf packs of Ogeechee tupelo (*Nyssa ogeche*), swamp tupelo (*N. biflora*), and bald cypress (*Taxodium distichum*) were incubated in a third-order stream reach and a 5th-order riparian swamp in an intermittent blackwater river within Georgia's coastal plain. Litter was analyzed for differences in microbial respiration and biomass, macroinvertebrate biomass, and changes in structural compound (lignin, cellulose, hemicellulose) content before and after a prolonged dry period. *N. ogeche*, with lowest initial concentrations of lignin, supported highest microbial biomass and respiration, although microbial biomass changed significantly over time and declined as the lignocellulose index (LCI) of litter increased. Macroinvertebrate shredder biomass was higher on *N. ogeche* litter, and was positively correlated with breakdown rates. Because macroinvertebrate biomass is twice as high in the swamp than in the stream, breakdown rates of labile litter were also twice as high. The initially high oxygen demand exerted by labile litter is ultimately diminished by rapid leaf mass loss due to the activity of microbes and macroinvertebrates. Although labile leaf litter initially supports

larger populations of microorganisms, it breaks down quickly, and recalcitrant litter has the potential to have greater prolonged ecosystem effects in reducing dissolved oxygen levels.

Introduction

The capacity of leaf litter derived from different riparian tree species to take up oxygen in aquatic ecosystems may influence ecosystem metabolism and system-wide oxygen availability. Allochthonous organic matter dominates ecosystem metabolism and is the primary basal resource in blackwater streams and rivers (Meyer and Edwards 1990). One of the largest inputs of allochthonous organic matter in forested streams is leaf litter, and differences in microbial biomass (Suberkropp and Klug 1976) and respiration and breakdown rates (Tank et al. 1993, Stelzer et al. 2003) among leaf litter species have been related to initial concentrations of leaf nutrients and recalcitrant structural compounds such as lignin (Melillo et al. 1982, Ardón and Pringle 2008). However, it has also been suggested that chemical differences among leaf litter species may become less pronounced over time as labile fractions of litter are selectively consumed and recalcitrant portions are left behind (Moore et al. 2004). Two different mechanisms of leaf breakdown have been proposed, in which recalcitrant structural compounds such as lignin are not broken down until more labile cellulose and hemicellulose have been consumed (Moorhead and Sinsabaugh 2006), or conversely, a higher concentration of labile structural compounds may enhance breakdown of lignin (Klotzbücher et al. 2011). A potentially useful metric for quantifying the effects of structural compounds on organic matter processing and microbial dynamics is the lignocellulose index (LCI), which is the ratio of lignin to the sum of all structural compounds (lignin + holocellulose) in the leaf litter (Melillo et al. 1989).

Blackwater streams are dynamic ecosystems experiencing high temperatures, reduced flow, and low dissolved oxygen (DO) during summer months (Meyer 1992). Leaf litter in all rivers and streams are subjected to a changing suite of abiotic and biotic factors (i.e. seasonal temperature regimes, biotic community shifts), but the repeated desiccation and re-wetting of leaf litter that occurs in many intermittent blackwater streams may affect organic matter processing in unique ways (Larned et al. 2010). As leaf litter breakdown may span multiple dry and wet periods, terrestrial and aquatic decomposer communities may play equally important roles in determining leaf litter effects on ecosystem metabolism.

Here we compare the uptake of oxygen by three leaf litter species over more than one year of incubation in an intermittent blackwater river. This incubation period spanned a period of complete natural drying of the river basin, allowing us to examine litter species effects before, during, and after the drying period. Our main objectives were to: (1) determine how different leaf litter species contribute to oxygen demand in blackwater streams; (2) determine the relative contributions of fungi, bacteria and macroinvertebrates to differences in leaf litter oxygen demand among tree species; and (3) to determine the effects of structural chemistry on litter associated oxygen demand and litter breakdown. We hypothesized that: (1) oxygen uptake rates would differ among leaf litter species; (2) differences in fungal and bacterial biomass would explain differences in oxygen uptake rates per gram, but leaf-shredding macroinvertebrate biomass would be a correlate of litter mass loss; and (3) increasing LCI would have negative effects on microbial biomass and respiration, as higher concentrations of structural compounds would limit the ability of microorganisms to process carbon.

Methods

Study site – This study was conducted in heavily forested third- and fifth-order reaches of the Little River, a blackwater river with its headwaters near Ashburn, Georgia, USA, draining the Atlantic coastal plain of North America. The two reaches, flowing through Turner and Tift counties, respectively, are contained within the Little River Experimental Watershed (LREW). Located in the headwaters of the upper Suwannee River basin, the 33,400-ha LREW was equipped in the 1960's and 1970's for continuous rainfall and stream flow measurements by the Southeast Watershed Research Laboratory (SEWRL) of the United States Department of Agriculture's Agricultural Research Service (USDA-ARS). Currently, the LREW has nine nested gauged sub-watersheds ranging in size from 260 to 33,400 ha. Detailed records of stream flow, nutrient concentrations, and DO concentrations are regularly collected in each sub-watershed (hourly, daily or weekly depending on sub-watershed and type of data collected). The third-order stream reach (31º41'32"N, 83º42'09"W, hereafter referred to as "stream") drains a 2,200 ha catchment, and meanders through a second-growth forest floodplain. Low-oxygen events occur every spring and summer, but oxygen concentrations rarely approach zero, and infrequently drop below 4 mg L^{-1} (Fig. 3.1). The fifth-order reach (31º28'54"N, 83º35'03"W, hereafter referred to as "swamp") drains a 33,400 ha catchment, and is often referred to as an in-stream swamp; the channel widens to roughly 300 meters, and water velocity is often very low. Low DO events are recurring and DO concentrations usually fall below 1 mg L^{-1} by April (Fig. 3.1). Both reaches dry completely, nearly every summer and fall. Summary chemical and physical variables are shown in Table 3.1.

Field procedures – We examined microbial respiration and biomass, breakdown, structural chemistry, and macroinvertebrate biomass associated with decomposing leaf litter of

three common southeastern coastal plain tree species differing in initial litter chemistry (Table 3.2). The three species selected were swamp tupelo (*Nyssa biflora* Walter), Ogeechee tupelo (*N. ogeche* Bartr. ex Marsh.), and bald cypress (*Taxodium distichum* [L.] Rich.). Between 2007 and 2008, ten-gram single-species leaf litter bags were incubated in the stream and swamp study reaches. Leaf litter from each species was collected immediately after abscission, air-dried in the laboratory, and placed into plastic mesh pecan bags (19.1 cm × 38.1 cm, 5 × 5 mm mesh; Cady Industries Inc., Georgia) following methods described by (Benfield, 1996). Leaf litter bags were grouped in arrays affixed to PVC tubing within the basin of the stream channel. Each array consisted of three leaf litter bags, with each bag containing litter from a different tree species. Leaf litter bags were organized into a randomized complete block design, with arrays grouped into blocks based on longitudinal distance downstream in the stream channel, and lateral distance (east-west) across the swamp channel. Five bags of each leaf litter species treatment (one from each block) were removed from the stream on each sampling date.

In situ rates of microbial respiration were estimated from oxygen uptake using methods described by Suberkropp et al. (2010). Briefly, after a leaf pack was removed from the stream, ten 17-mm-diameter disks (or 1-cm sections of *T. distichum*) were cut from a single species and immediately enclosed into a 26-mL respiration chamber containing unfiltered stream water. Changes in DO concentrations within chambers were measured every 5 minutes for 30 minutes using a YSI 5100 Dissolved Oxygen Meter (Yellow Springs, OH, U.S.A.). All measurements were conducted at ambient stream water temperatures in darkness. Oxygen uptake rate was determined by the slope of the regression of DO concentrations versus time, minus a control slope using stream water alone. Following respiration measurements, leaf discs were placed into labeled foil packets, placed on ice, transported to the laboratory.

Additional leaf discs were simultaneously cut and preserved in the field for estimation of bacterial and fungal biomass (ergosterol). Leaf disks for bacterial biomass (five 4-mm-diameter disks) were placed into sterile 15-ml polystyrene tubes containing 5 ml sterile-filtered 2% phosphate buffered formalin, and placed on ice. Leaf disks for fungal biomass (five 12-mm-diameter disks) were placed into clean 20-ml plastic scintillation vials and frozen on dry ice. Samples were immediately transported to the laboratory. Bacterial samples were stored at 4°C, and fungal samples were lyophilized, weighed, and stored in 5 mL HPLC-grade methanol at -20˚C, until analysis. The remaining litter bag material was placed into clean, individually-labeled resealable plastic bags filled with stream water, and immediately placed on ice and transported to the laboratory for further processing.

Laboratory procedures – In the laboratory, leaf disks from respiration measurements were oven-dried for one week at 60ºC, weighed, and a sub-sample combusted at 500ºC to determine leaf litter ash-free dry mass (AFDM). The remaining leaf material within litter bags was rinsed over a sieve (1 mm mesh size) to remove macroinvertebrates and other foreign material. Invertebrates were preserved in 80% ethanol and later identified to the lowest possible taxonomic level (usually genus, Appendix C). Invertebrates were measured to determine dry mass from published length-mass regressions (Benke et al. 1999), and separated into functional feeding groups according to Merritt et al. (2008).

Leaves were dried for one week at 60ºC, weighed, and a sub-sample combusted at 500ºC to determine AFDM. Breakdown rate (k) was determined from the slope of the natural log of mass remaining versus time in days (Webster and Benfield 1986). Litter chemistry was analyzed with remaining oven-dried leaf material from litterbags. Litter was ground to a powder capable of passing through a 250-µm mesh. C and N concentrations were analyzed using a Carlo Erba

1500N CHN Analyzer (Carlo Erba, Milan, Italy). Cellulose, hemicellulose, and lignin concentrations were determined using an Ankom A200 Fiber Analyzer (Ankom, Macedon, New York, USA) according to established protocols from the manufacture. For phosphorus content analysis, 10 mg of ground dried litter was weighed, combusted at 500°C, extracted with 0.25 mL of aqua regia, and diluted with 10 mL of deionzied water. Phosphorus was measured from diluted extracts on a colorimetric analyzer (Alpkem 300 Series Autoanalyzer, ortho-PO4 manifold, EPA method 365.1, APHA (1999)).

Fungal biomass was estimated from ergosterol concentrations in preserved leaf litter samples. Ergosterol was extracted in alcoholic KOH (0.8% KOH in methanol, total extraction volume 10 ml) for 30 minutes at 80ºC in tightly capped tubes with constant stirring. The resultant crude extract was partially cleaned by solid phase extraction (Gessner and Schmitt 1996), and ergosterol quantified by high-pressure liquid chromatography (HPLC). A LichroSpher 100 RP-18 column (0.46 × 25 cm, Merck) maintained at 40°C in a Shimadzu column oven (CTO-10AS) and connected to a Shimadzu autosampler (SIL-10AD) and Shimadzu liquid chromatograph system (Pumps LC-10AT, Controller SCL-10A) was used for separation and analysis. The mobile phase was HPLC grade methanol at a flow rate of 1.5 ml min–1. Ergosterol was detected at 282 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time = ca. 8 min), and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical).

Bacterial biomass was estimated using epifluorescence direct count microscopy and analysis of captured microscope images. Bacteria attached to preserved leaf litter samples were removed by ultrasonication for 1.5 minutes using a Bransonic 150 probe sonicator, with sample tubes placed on ice to prevent excessive sample heating (Buesing and Gessner 2002). Samples were subsequently centrifuged at 800g for 1 minute in order to eliminate large suspended

particles and facilitate image analysis. The supernatant from each tube was transferred to a new sterile 15-ml polystyrene tube and vortexed for 15 s to ensure homogenization of the sample. From each sample, 1 ml of formalin-preserved bacteria was filtered through a 0.2-µm Anodisc filter and stained with SYBR Gold (Patel et al. 2007). Twenty images were randomly captured from each filter at 1000X magnification using an Olympus BH-2 microscope and an Olympus Qcolor 3 digital camera (Olympus ®, Melville, NY), and analyzed using the MatLab (v 7.9) image processing toolbox. Cell counts were used to measure bacterial concentration. Biovolume estimates (μm^3) were calculated from length (l) and width (w) measurements and converted to bacterial biomass following published protocols (First and Hollibaugh 2008).

Statistical analysis

Microbial respiration among litter species was expressed as O_2 uptake per gram of litter and also per leaf pack. When expressed per pack, the average O_2 uptake rate for a given litter species was multiplied by the grams of organic matter remaining for the same litter species on the same date. The effects of leaf litter species and incubation length (days) on microbial respiration, fungal biomass and bacterial biomass were analyzed with analysis of covariance (ANCOVA), using PROC GLM at $\alpha = 0.05$ in SAS version 9.1 (SAS Institute Inc., Cary, USA). The effects of LCI on fungal, bacterial, and macroinvertebrate biomass were analyzed with analysis of covariance (ANCOVA), also using PROC GLM at $\alpha = 0.05$ in SAS version 9.1. If the assumption of equal covariate slopes was violated (in the case of fungal biomass), the analysis was split among tree species. Multivariate analysis of variance (MANOVA) was used to examine the effects of leaf litter species on functional feeding group (collector-gatherer, collector-filterer, predator, scraper, and shredder) biomass in leaf litter. Planned pairwise comparisons (Bonferroni method, $\alpha = 0.05$, Milliken and Johnson 1992) among leaf litter species were conducted when

main effects were significant. Data were log-transformed whenever necessary to meet the assumptions of normality and homoskedasticity.

To determine factors explaining microbial respiration (O_2 uptake) in leaf litter, as well as macroinvertebrate effects on leaf litter breakdown rates, we compared candidate multiple regression models using Akaike's Information Criterion (AIC) and the information theoretic approach (Burnham and Anderson 2002) to select the most plausible models based on lowest AIC_c (AIC corrected for small sample size, selected based on lowest Mallows' C_p) using PROC REG at $\alpha = 0.05$ in SAS version 9.1 (SAS Institute Inc., Cary, USA). Differences between a candidate model's AIC_c and that of the top model (Δ_i), as well as Akaike weights (w_i), were calculated for all candidate models with Δ_i not greater than ten. When summed parameter importance weights were determined, calculations were limited to a confidence set that included all candidate models with a w_i of at least 5% of the top model's w_i. For regression models dealing with respiration, samples of microbial biomass and measurements of microbial respiration were treated as subsamples and averaged per litter species on each sampling date. This was deemed necessary because samples of microbial biomass and respiration were taken from separate regions of leaf litter, and distributions of microorganisms on leaf litter are patchy (Shearer and Lane 1983). Any explanatory variable not linearly correlated with the response variable was excluded from candidate models. Explanatory variables were tested for multicollinearity by examining Pearson's correlation coefficient matrices and calculating variance inflation factors (VIF). The average VIF in final models did not exceed the total number of explanatory variables (Neter et al. 2004), nor did it exceed 2 in any single parameter.

Results

Microbial respiration and biomass – In the swamp, oxygen uptake differed significantly over time ($F_{1,111}$=7.70, p<0.01, Fig. 3.2A) and among the three leaf litter species ($F_{2,111}$=9.59, p<0.0001, Fig. 3.2A). Microbial oxygen uptake per gram of *N. ogeche* litter was consistently higher than uptake rates per gram of *N. biflora* or *T. distichum* litter throughout the study period (Bonferroni, all p<0.0001, Fig. 3.2A). In contrast, oxygen uptake rates per leaf pack of *N. ogeche* were generally lower than per-leaf-pack oxygen uptake rates of the other two leaf litter species after longer incubations (Fig. 3.2C).

In the stream, there was a significant interaction effect of leaf litter species and incubation time on oxygen uptake rates per gram of litter ($F_{2,83}$=3.92, p<0.05, Fig. 3.2B). Oxygen uptake was initially highest per gram of *T. distichum*, but was later higher on *N. ogeche* litter than on the other two leaf litter species (Fig. 3.2B). Oxygen uptake per leaf pack was initially highest in T. distichum litter, then on *N. ogeche*, and finally on *T. distichum* and *N. biflora*. In both the swamp and the stream, which leaf litter species supported highest microbial oxygen uptake per pack was primarily due to differences in breakdown rates (k) (Table 3.2). The breakdown rate of *N. ogeche* was more than 3× and 2× higher than that of *N. biflora* or *T. distichum* in the stream and swamp, respectively. Breakdown rates were also higher in the swamp than in the stream, with rates of *N. ogeche*, *N. biflora*, and *T. distichum* being 2.11, 1.41, and 1.29× higher during the first wet period in the swamp, respectively (Table 3.2).

Fungal biomass differed significantly among leaf litter species, but relative differences among species changed significantly over time, in both the swamp (species×time, $F_{16,104}$ = 13.11, p < 0.0001, Fig. 3.3) and in the stream (species×time, $F_{12,104}$ = 6.29, p < 0.0001, Fig. 3.3). In the swamp, fungal biomass was initially higher in *T. distichum* litter than in *N. ogeche* litter

(Bonferroni, $p < 0.0001$), and remained higher than the other leaf litter species through 79 days of incubation, although differences were not statistically significant. *N. ogeche* litter exhibited the highest fungal biomass concentrations from 98 days of incubation until the end of the study, supporting significantly higher fungal biomass than *N. biflora* after 119 days of incubation (Bonferroni, $p < .005$), and significantly higher biomass than both *N. biflora* and *T. distichum* from the dry period until the end of the study (Bonferroni, all $p < 0.0001$). In the stream, differences among litter species and temporal changes in fungal biomass were similar to those observed in the swamp (Fig. 3.3). Fungal biomass was initially highest in *T. distichum* litter, with *N. ogeche* generally supporting intermediate levels of fungal biomass during early stages of breakdown, and then gradually supporting more fungal biomass than *T. distichum* and *N. biflora* during later stages of breakdown. No statistically significant differences were observed until the final post-dry period sampling date, when *N. ogeche* litter supported significantly higher fungal biomass than the other two leaf litter species (Bonferroni, all $p < 0.05$)

Bacterial biomass was affected by an interaction between leaf litter species and incubation time in the swamp (species×time, $F_{14,48} = 2.36$, $p < 0.01$, Fig. 3.4), with N. ogeche litter generally supporting highest bacterial biomass among the three tree species (Bonferroni, $p < 0.005$). Bacterial biomass on *N. ogeche* litter began to decline later in the first wet period, while biomass on the other two litter species continued to increase. During the dry period bacterial biomass on all three litter species significantly decreased, but increased after inundation during the second wet period, when *N. biflora* supported highest bacterial biomass. In the stream, bacterial biomass differed significantly among litter species ($F_{2,36}= 62.16$, $p < 0.0001$, Fig. 3.4) and increased over time ($F_{5,36}= 13.47$, $p < 0.0001$, Fig. 3.4), with bacterial biomass significantly

different among all three litter species and consistently highest on *N. ogeche*, intermediate on *N. biflora*, and lowest on *T. distichum* litter (Bonferroni, all $p < 0.0001$).

Among candidate regression models including all possible combinations of three parameters, the combination of fungal biomass, bacterial biomass, and temperature best explained oxygen uptake rates per gram of leaf litter in both the swamp and in the stream (Table 3.3). A greater proportion of the variability in oxygen uptake rates was explained in the stream ($F_{3,13} = 31.40$, $r^2_{adj} = 0.85$, $p < 0.0001$) than in the swamp ($F_{3,18} = 11.12$, $r^2_{adj} = 0.59$, $p < 0.0005$). Fungi and bacteria were equally important (0.85) parameters explaining oxygen uptake in the swamp, but fungi and temperature had slightly greater importance (0.99) than bacteria (0.74) in the stream, with some support for a model excluding bacterial biomass.

Macroinvertebrate consumers – Macroinvertebrate biomass was generally higher in the swamp than in the stream (Tables 3.4-3.5, Fig. 3.5). In the swamp, shredder biomass differed significantly among leaf litter species and changed significantly over time (Table 3.4), with *N. ogeche* having the highest shredder biomass per gram of litter, *T. distichum* having the lowest, and *N. sylvatica* supporting intermediate levels (Bonferroni, all $p < 0.01$). Shredder biomass per leaf pack followed the same patterns as those observed per gram of litter, but differences were not statistically significant ($F_{2,42} = 2.68$, $p = 0.0804$). Shredder biomass generally increased through time during each wet period in the swamp, whether expressed per gram of litter (Table 3.4) or per leaf pack ($F_{6,42} = 21.44$, $p < 0.0001$). In both cases, biomass was highest on the last date before drying in each wet period (Bonferroni, all $p < 0.05$).

In the stream, shredder biomass was affected by an interaction between leaf litter species and days (Table 3.5, Fig. 3.5). Differences in shredder biomass per gram of litter were statistically significant only on the final sampling date, when *N. ogeche* litter contained

significantly higher shredder biomass than *N. biflora* or *T. distichum* (Bonferroni, all $p < 0.001$). There were no significant differences in shredder biomass per pack among the three litter species in the stream. Shredder and collector-gatherer biomass were significantly correlated with litter breakdown rates in both the swamp and in the stream (Table 3.6), with some support for an additional effect of scrapers on mass loss rates in the swamp. Predator biomass in the stream was often dominated by terrestrial soil centipedes during the second wet period. When encountered, these were alive and active in litter bags far from the stream edge and 10-30 centimeters under water. A list of all organisms encountered in litterbags is provided in Appendix C.

Structural chemistry and lignocellulose index effects – Leaf litter species differed significantly in initial concentration of structural compounds (Table 3.2). The breakdown rate of each structural compound was determined by a significant interaction between type (cellulose, hemicellulose, lignin) and leaf litter species in the swamp ($F_{6,21} = 3.19$, $p < 0.05$, Fig. 3.6A) and in the stream ($F_{6,24} = 3.35$, $p < 0.05$, Fig. 3.6B). The initial lignocellulose index (LCI) differed significantly among leaf litter species (Table 3.2), and increased over time at different rates among litter species in both the swamp (ANCOVA, days×species, $F_{2,93} = 10.33$, $p < 0.0001$, Fig. 3.7A) and in the stream (ANCOVA, days×species, $F_{2,72} = 6.43$, $p < 0.005$, Fig. 3.7E). As a covariate with leaf litter species, LCI did not have a significant effect on microbial oxygen uptake in either site or on bacterial biomass in the swamp, but it was positively correlated with bacterial biomass in the stream (ANCOVA, $F_{1,72} = 31.27$, $p < 0.0001$), and had a significant negative effect on shredder biomass in the swamp (ANCOVA, $F_{1,39} = 4.33$, $p < 0.05$) and in the stream (ANCOVA, $F_{1,29} = 6.49$, $p < 0.05$). The strongest negative effects of LCI were on fungi, but these differed among species in both the swamp (ANCOVA, species×LCI, $F_{2,78}=8.72$, $p < 0.0005$) and in the stream (ANCOVA, species×LCI, $F_{2,57}=10.49$, $p < 0.0005$). Separate

regressions for each litter species (Figs. 3.7B-D, 3.7F-H) revealed strongest negative effects of LCI on fungal biomass in *T. distichum* litter in both the swamp ($t_{1,26}$ = -11.53, r^2_{adj} = 0.83, p < 0.0001, Fig. 3.7D) and stream ($t_{1,19}$ = -9.72, r^2_{adj} = 0.82, p < 0.0001, Fig. 3.7H). In the stream, significant negative correlations were also observed between LCI and fungi in *N. ogeche* ($t_{1,19}$ = -3.15, r^2_{adj} = 0.37, p < 0.005, Fig. 3.7F) and *N. sylvatica* litter ($t_{1,19}$ = -3.10, r^2_{adj} = 0.30, p < 0.01, Fig. 3.7G), but these relationships did not exist for either species of *Nyssa* litter in the swamp (Figs. 3.7B-C).

Discussion

Our findings illustrate a complex interaction between litter chemistry and numerous members of the decomposer guild to affect leaf-litter-generated oxygen demand in blackwater swamps and streams. While more labile litter species initially support higher microbial biomass and therefore higher oxygen uptake rates, they are broken down more rapidly by microbial decomposition and macroinvertebrate consumption. This decreases available substrate for microbial growth, thereby reducing the amount of oxygen consumed by labile litter relative to recalcitrant litter over longer incubations. Furthermore, as leaf litter quality, and carbon quality in particular, is reduced over time, oxygen uptake rates per gram of litter are also reduced. Fungal biomass was negatively correlated to the lignocellulose index (LCI) of litter, illustrating a critical link between carbon quality and microbial oxygen uptake.

Differences in fungal and bacterial biomass among litter species explained much of the variation in oxygen uptake rates per gram of litter (Table 3.3). However, although oxygen uptake rates were higher in the swamp, correlation with fungal biomass was lower. This may be partially due to lower oxygen concentrations in the swamp (Fig. 3.1). Oxygen from the water

column must first pass through bacterial biofilms before reaching fungi. Living primarily in the leaf interior (Newell et al. 1996, Gessner et al. 2007), between two diffusive boundary layers created by surface biofilms, fungi may be at a competitive disadvantage in oxygen uptake. Diffusive boundary layer thickness, biofilm thickness, and biofilm bacterial densities increase as water velocity decreases (Jorgensen and Revsbech 1985, Battin et al. 2003), and anoxic conditions have been measured under low flow conditions at the base of biofilms less than 0.5 mm in thickness (Jorgensen and Revsbech 1985, Glud et al. 1998). Water velocities were lower (Fig. 3.1) and bacterial biomass was generally higher (Fig. 3.4) in the swamp, potentially reflecting thicker biofilms and potentially reduced passage of oxygen to leaf interiors. Additionally, as oxygen availability decreases, fungal chitin:biomass and ergosterol:biomass ratios are known to vary considerably (Sharma et al. 1977, Charcosset and Chauvet 2001). While one conversion factor (5.5) was used to convert ergosterol to fungal biomass on all sampling dates, the true ratio of ergosterol:biomass may have changed over time depending on oxygen availability, thus potentially reducing the correlation between oxygen uptake and apparent fungal biomass.

The reduced role that labile litter plays in oxygen demand after longer incubations (Fig. 3.2, bottom) is partially caused by a loss of mass and substrate for microbial growth and respiration. This is due to enhanced carbon loss via higher microbial respiration and CO_2 release from labile leaf litter, but is also affected by preferential consumption of labile leaf litter by macroinvertebrates (Fig. 3.5), thereby reducing substrate for microbial growth and respiration. Material ingested by larger consumers has not entirely left the aquatic system, but instead fuels the metabolic requirements of larger detritivores, which pose an additional source of oxygen demand. The export of carbon and nutrients to riparian zones through insect emergence can be

quite high (Baxter et al. 2005), potentially lowering biological oxygen demand as insects move to the terrestrial environment. However, the shredder community in the swamp was dominated by crustaceans, which complete their life cycles in the aquatic environment. Larger consumers may have lower mass-specific metabolic rates than microorganisms (Brown et al. 2004), but while this represents reduced oxygen consumption, it also reduces CO_2 release from the system and may ultimately retain more carbon in the long term, if emergence rates are low.

Our results did not agree well with the theoretical model of lignin breakdown proposed by (Moorhead and Sinsabaugh 2006), which suggests that lignin decays very slowly when holocellulose is abundant. Instead, lignin breakdown rates in both the swamp and in the stream were significantly faster in *N. ogeche* litter, which had lowest initial lignin content, and were slowest in *T. distichum* litter, which had the highest lignin content (Table 3.2, Fig. 3.6). This falls more in line with the "co-metabolic lignin degradation" mechanism proposed by (Klotzbücher et al. 2011), in which higher availability of more decomposable cellulose and hemicellulose facilitate lignin breakdown. Although initial N and P content was higher in *T. distichum* litter than in *N. ogeche*, lignin was not more readily degraded and breakdown proceeded more slowly. Instead, lignin content relative to content of nutrients or holocellulose appeared to determine microbial biomass. While ratios of leaf litter lignin:N and the LCI predicted breakdown rates fairly well, ratios of C:N, C:P, and N:P were less useful. Furthermore, although the swamp had higher water column concentrations of N and P (Table 3.1), lignin broke down more slowly there than in the stream (Fig. 3.6). Inconsistent interactions between lignin breakdown and N availability have also been demonstrated by Moorehead and Sinsabaugh 2006. Much of the breakdown of structural compounds in the stream took place during the dry period. This is not surprising, as lignin degradation requires oxygen (Bayer et al. 2006). Leaf litter LCI increased

dramatically during the first half of the dry period in the stream, but the LCI declined during the latter half of the dry period. This may have been caused by enhanced microbial degradation of lignin Brought about by greater oxygen availability during the dry period.

Although labile leaf litter initially supports larger populations of microorganisms and exerts higher oxygen demand, recalcitrant litter has the potential to have greater long-lasting ecosystem effects on metabolism, by way of sheer resilience. Microbial biomass is intimately linked to carbon quality, and is therefore affected by the initial quality of leaf litter as it enters a system, and by the changes in quality that occur throughout the breakdown process. Understanding these fine scale processes may ultimately elucidate how blackwater river systems work on larger scales. Since microorganisms play a key role in oxygen uptake in aquatic ecosystems, perturbations affecting carbon quality (i.e. logging, shifting forest composition) could have profound consequences for oxygen dynamics. Because leaf litter species consume oxygen at significantly different rates, our findings further suggest that forest composition may be able to affect benthic oxygen demand on larger scales in blackwater rivers.

Figure 3.2 illustrates the effects of forest composition and also the importance of external processes (such as macroinvertebrate feeding) on the amount of oxygen demand generated by leaf-litter-associated microorganisms. Oxygen uptake rates per litter pack are shown in the bottom panels, which is useful in comparing the amount of oxygen demand generated by different leaf litter species, and the ways in which demand changes over time. For example, if two river reaches received equal quantities of litter, but one received leaf litter from *N. ogeche* and the other received leaf litter from either *N. sylvatica* or *T. distichum*, the N. ogeche-dominated reach may be initially lower in oxygen due to higher rates of microbial respiration. However, the organic carbon in leaf litter is gradually converted to CO_2 and exits the system, or

it is consumed by macroinvertebrates. Therefore, as more time progresses after leaf fall, leaf-litter-generated oxygen demand in the *N. ogeche*-dominated reach will gradually decline. However, as observed in the stream reach (right panels), a lack of macroinvertebrates may slow the degree to which leaf litter is converted to fine particulate organic matter, therefore extending the effects of labile leaf litter (relative to recalcitrant *T. distichum* leaf litter) through time.

These complex interactions provide a challenge to policy makers. Leaf litter breakdown rates are incorporated into environmental fluid dynamics code (EFDC) models that are used to predict what natural DO concentrations should be in an unimpacted blackwater river. However, due to the relatively small number of litter breakdown measurements in blackwater rivers, models are often parameterized with values from unrelated tree species in ecosystems far-removed from the study site. Here we have demonstrated that leaf litter breakdown rates within a blackwater river differ significantly depending on the tree species from which they are derived, but also that they differ substantially for a single tree species in two different reaches of the same river. These differences may be due in large part to differences between sites in the relative abundance (or lack thereof) of leaf-shredding macroinvertebrates. A clearer understanding of processing rates must be incorporated into site-specific target oxygen minima. Otherwise, greater uncertainty should be incorporated into a range of target DO minima within a site.

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Table 3.1: Summary physical and chemical data for third order (stream) and fifth order (swamp) study reaches within the Little River Experimental Watershed during 2007-2008.

	Stream	**Swamp**
DOC (mg L^{-1})	11.25 ± 0.68	22.89 ± 1.47
SRP (µg L^{-1})	9.83 ± 4.09	22.67 ± 1.47
Total P (µg L^{-1})	46.50 ± 10.38	79.59 ± 28.38
NO_3^- (µg L^{-1})	27.27 ± 8.30	33.07 ± 10.58
NH_4^+ (µg L^{-1})	28.13 ± 7.27	36.54 ± 9.00
Total N (µg L^{-1})	1199.47 ± 255.13	969.63 ± 249.00
pH	5.76 ± 0.08	6.11 ± 0.04
Discharge (L s^{-1})	353.29 ± 40.34	3343.06 ± 445.66

Table 3.2: Mean breakdown rate during the first wet period, initial percent concentration of leaf litter structural compounds and nutrients, and lignocellulosic index. Standard errors (± 1) are provided in parentheses. n = 5 for all parameters except percent nutrient concentration (n = 3).

Litter species	K_{swamp}	K_{stream}	lignin	hemicellulose	cellulose	C	N	P	LCI
N. ogeche	0.0214 (0.0010)	0.0101 (0.0007)	8.06 (0.19)	11.60 (0.32)	19.74 (0.37)	50.25 (1.24)	0.81 (0.043)	0.029 (0.0009)	0.20 (0.003)
N. biflora	0.0066 (0.0002)	0.0047 (0.00003)	11.34 (0.43)	11.32 (0.28)	17.66 (0.44)	51.22 (0.18)	1.05 (0.008)	0.035 (0.0034)	0.28 (0.010)
T. distichum	0.0062 (0.0002)	0.0048 (0.00008)	16.69 (0.63)	10.59 (0.21)	25.11 (0.53)	51.59 (0.25)	0.95 (0.021)	0.047 (0.0013)	0.32 (0.010)

Table 3.3: Comparison of candidate multiple regression models explaining variation in oxygen uptake generated by leaf litter. *K* is the number of parameters in the multiple regression model (including y-intercept and error), C_p is Mallows' C_p, AIC_c is Akaike's second-order information criterion (corrected for small sample size), Δ_i is the difference between the candidate model and the best model's AIC_c, *L* is the likelihood value of each model, and w_i is the relative strength of evidence for each candidate model (between 0-1). Parameter importance weights are calculated as the sum of the values of w_i for all models containing the parameter of interest.

Swamp	***K***	C_p	AIC_c	Δ_i	***L***	w_i	r^2_{adj}
candidate model							
fungi, bacteria, temperature	5	4.00	-105.85		1.00	0.85	0.59
fungi, bacteria	4	6.48	-104.36	3.71	0.16	0.13	0.56
bacteria, temperature	4	12.00	-99.53	8.54	0.01	0.01	0.45
bacteria	3	14.52	-99.25	8.81	0.01	0.01	0.37

Stream	***K***	C_p	AIC_c	Δ_i	***L***	w_i	r^2_{adj}
candidate model							
fungi, bacteria, temperature	5	4.00	-88.36		1	0.74	0.85
fungi, temperature	4	7.82	-86.19	2.17	0.34	0.25	0.80

parameter	**fungi**	**bacteria**	**temp.**
importance weight (swamp)	0.98	0.98	0.85
importance weight (stream)	0.99	0.74	0.99

Table 3.4: Univariate and multivariate statistical results for leaf litter species and incubation time effects on macroinvertebrate functional feeding group (FFG) biomass in the swamp.

Swamp						
ANOVA FFG, factor	**Biomass (mg g^{-1} litter AFDM) average**	**range**	***df***	**MSE**	**F**	***p***
collector-filterer	7.41	0 – 274.69				
litter species			2	6.17	22.80	<0.0001
days			6	7.39	27.29	<0.0001
litter species × days			12	0.88	3.27	**<0.005**
error			28			
collector-gatherer	1.77	0 – 9.59				
litter species			2	4.47	33.57	<0.0001
days			6	1.35	10.15	<0.0001
litter species × days			12	0.28	2.11	**<0.05**
error			28			
predator	3.64	0 – 152.51				
litter species			2	6.17	1.48	<0.05
days			6	7.39	3.11	<0.0001
litter species × days			12	0.88	0.85	**<0.05**
error			28			
scraper	2.66	0 – 46.89				
litter species			2	2.98	22.80	<0.005
days			6	24.07	27.29	<0.0001
litter species × days			12	5.80	3.27	**<0.05**
error			28			
shredder	41.96	0.38 – 591.54				
litter species			2	21.16	57.85	**<0.0001**
days			6	7.41	20.27	**<0.0001**
litter species × days			12	0.55	1.50	ns
error			28			
total	57.44	0.75 – 674.65				
litter species			2	20.63	81.16	<0.0001
days			6	6.68	26.28	<0.0001
litter species × days			12	0.56	2.19	**<0.05**
error			28			
MANOVA factor			***df***	**Wilks' λ**	**F**	***p***
litter species			12, 74	0.083	15.17	<0.0001
days			36, 165	0.006	10.12	<0.0001
litter species × days			72, 207	0.057	1.99	<0.0001

Table 3.5: Univariate and multivariate statistical results for leaf litter species and incubation time effects on macroinvertebrate functional feeding group (FFG) biomass in the stream

Stream						
ANOVA FFG, factor	**Biomass (mg g^{-1} litter AFDM) average**	**range**	***df***	**MSE**	**F**	***p***
collector-filterer	0.02	0 – 0.50				
litter species			2	0.07	1.21	ns
days			5	0.04	0.73	ns
litter species × days			10	0.03	0.59	ns
error			28			
collector-gatherer	4.07	0.002 – 125.79				
litter species			2	0.79	4.02	<0.05
days			5	3.67	18.65	<0.0001
litter species × days			10	0.85	4.30	**<0.005**
error			28			
predator	7.15	0 - 279.65				
litter species			2	0.46	0.53	ns
days			5	1.14	1.33	ns
litter species × days			10	0.70	0.82	ns
error			28			
scraper	0.03	0 – 0.25				
litter species			2	0.005	0.46	ns
days			5	0.007	0.65	ns
litter species × days			10	0.007	0.66	ns
error			28			
shredder	3.28	0 – 54.09				
litter species			2	2.18	16.52	<0.0001
days			5	3.78	28.61	<0.0001
litter species × days			10	0.80	6.08	**<0.0001**
error			28			
total	14.55	0.44 – 303.41				
litter species			2	4.14	11.62	<0.0005
days			5	4.02	11.29	<0.0001
litter species × days			10	1.07	3.01	**<0.05**
error			28			
MANOVA factor			***df***	**Wilks' λ**	**F**	***p***
litter species			12, 44	0.285	3.20	<0.005
days			30, 90	0.016	5.42	<0.0001
litter species × days			60, 120	0.038	1.74	<0.01

Table 3.6: Comparison of candidate multiple regression models explaining variation in daily leaf litter mass loss rates. *K* is the number of parameters in the multiple regression model (including y-intercept and error), C_p is Mallows' C_p, AIC_c is Akaike's second-order information criterion (corrected for small sample size), Δ_i is the difference between the candidate model and the best model's AIC_c, *L* is the likelihood value of each model, and w_i is the relative strength of evidence for each candidate model (between 0-1). Parameter importance weights are calculated as the sum of w_i's for all models containing a given parameter.

Swamp	***K***	C_p	AIC_c	Δ_i	***L***	w_i	r^2_{adj}
candidate model							
shredders, collector-gatherers	4	2.61	-50.00		1.00	0.70	0.41
shredders, collector-gatherers, scrapers	5	4.00	-48.29	1.71	0.42	0.30	0.41

Stream	***K***	C_p	AIC_c	Δ_i	***L***	w_i	r^2_{adj}
candidate model							
shredders	3	0.64	-77.89		1.00	0.53	0.26
shredders, collector-gatherers	4	2.00	-76.19	1.70	0.43	0.23	0.25
shredders, scrapers	4	2.57	-75.58	2.31	0.31	0.17	0.24
shredders, collector-gatherers, scrapers	5	4.00	-73.68	4.21	0.12	0.07	0.24

parameter	**sh**	**c-g**	**sc**
importance weight (swamp)	1.21	1.21	0.36
importance weight (stream)	1.00	0.29	0.23

Figure 3.1: Discharge, dissolved oxygen, and sampling dates over time in the swamp and stream. Discharge is shown on the primary ordinate (note the difference in scale in top and bottom panels), and dissolved oxygen concentration is shown on the secondary ordinate. Sampling dates (incubation times in days) are indicated with arrows, with "0" indicating the date of leaf pack deployment.

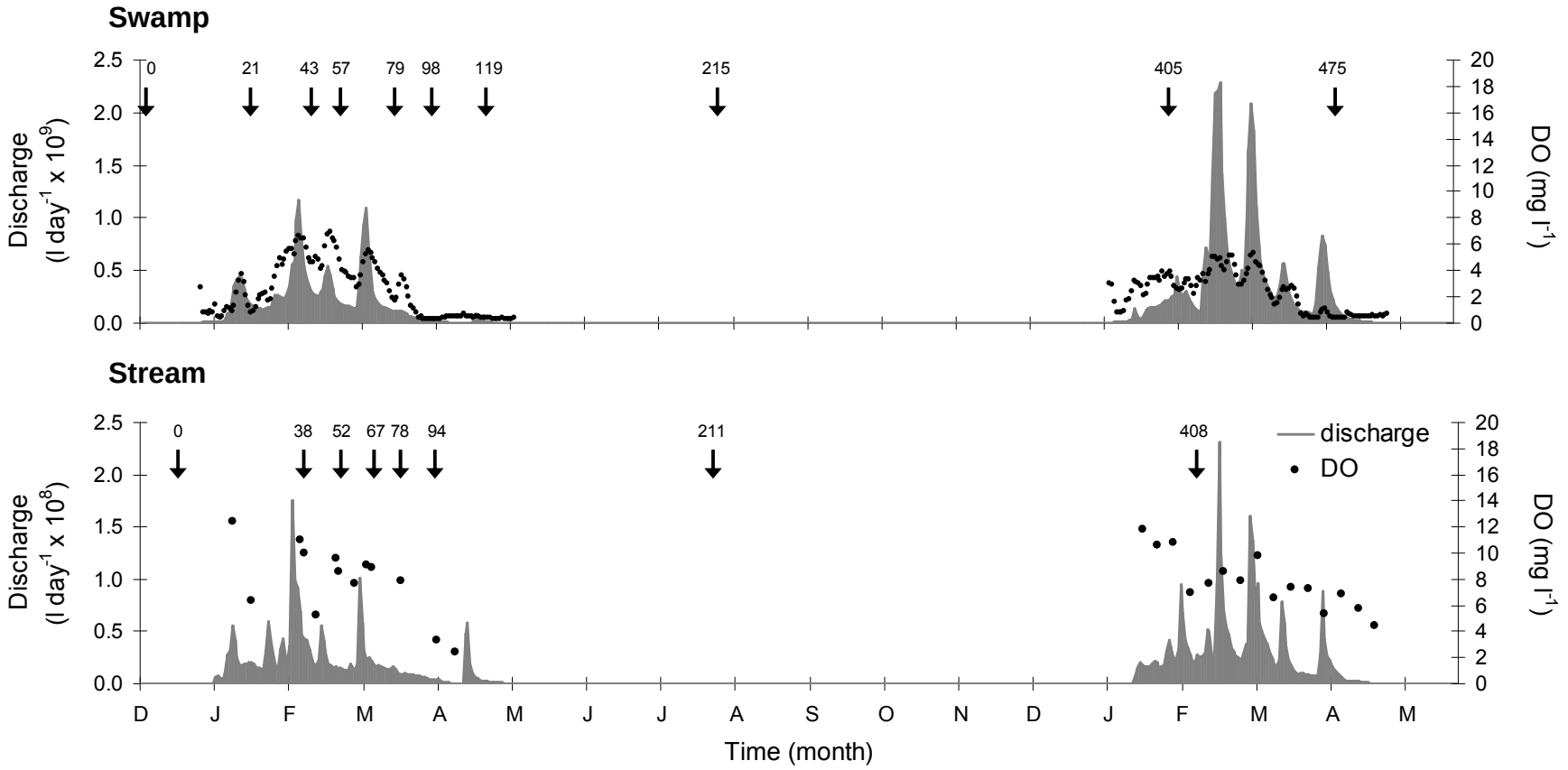

Swamp
Discharge (l day-1 x 10^9)
DO (mg l-1)
0
21
43
57
79
98
119
215
405
475
Stream
Discharge (l day-1 x 10^8)
DO (mg l-1)
0
38
52
67
78
94
211
408
discharge
DO
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
Time (month)

Figure 3.2: Leaf litter oxygen uptake over time in the swamp and stream, expressed per gram of leaf litter (top panels, with temperature on secondary ordinate) and per leaf pack (bottom panels). Different letters above columns indicate statistically significant differences among litter species within a sampling date.

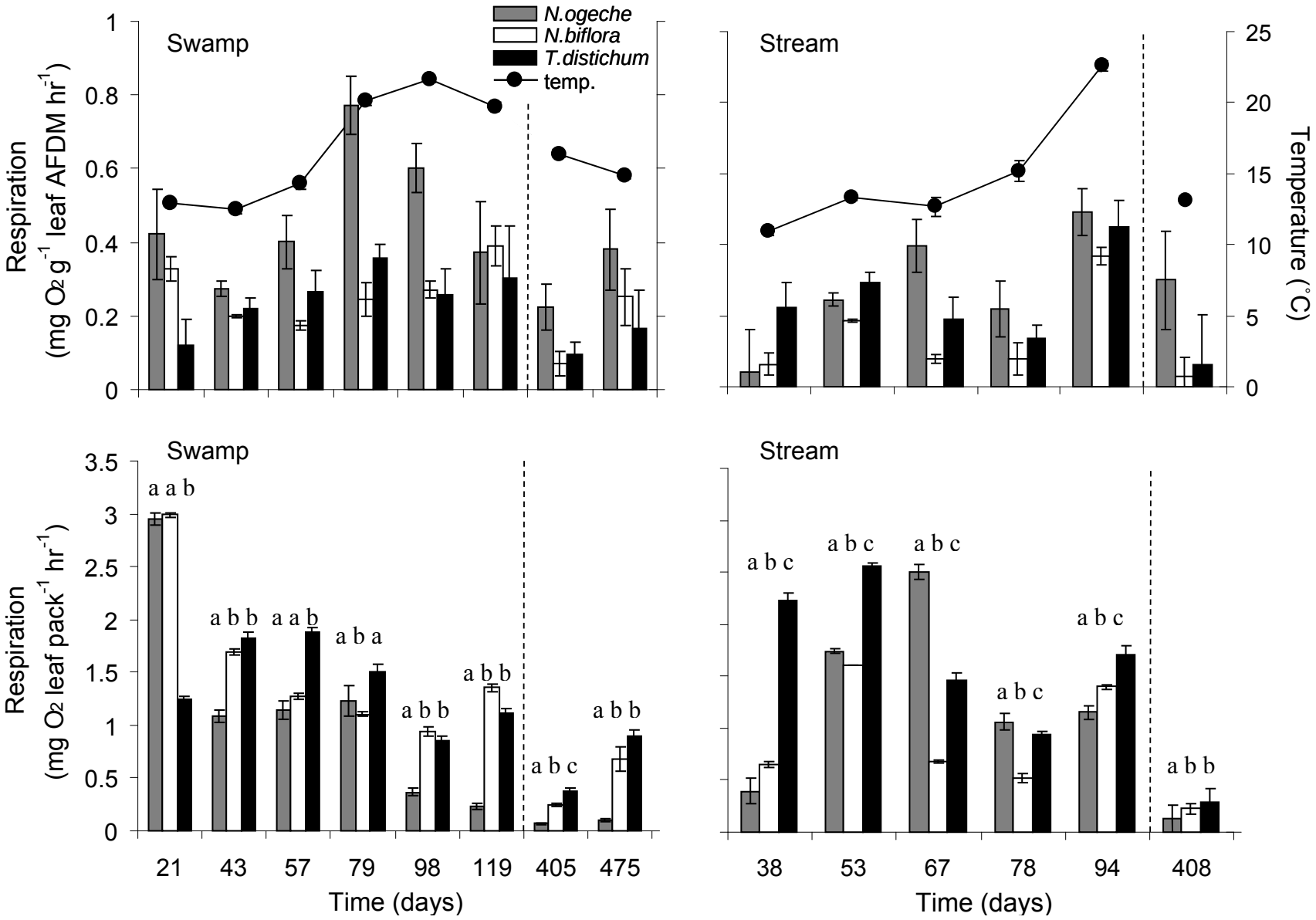

Swamp
Stream
N.ogeche
N.biflora
T.distichum
temp.
Respiration (mg O2 g-1 leaf AFDM hr-1)
Temperature (°C)
Respiration (mg O2 leaf pack-1 hr-1)
a a b
a b b
a a b
a b a
a b b
a b b
a b c
a b c
a b c
a b c
a b c
a b c
a b c
a b b
21
43
57
79
98
119
405
475
38
53
67
78
94
408
Time (days)
Time (days)

Figure 3.3: Leaf litter fungal biomass over time in the swamp and stream. Dashed vertical lines indicate the beginning and end of the dry period.

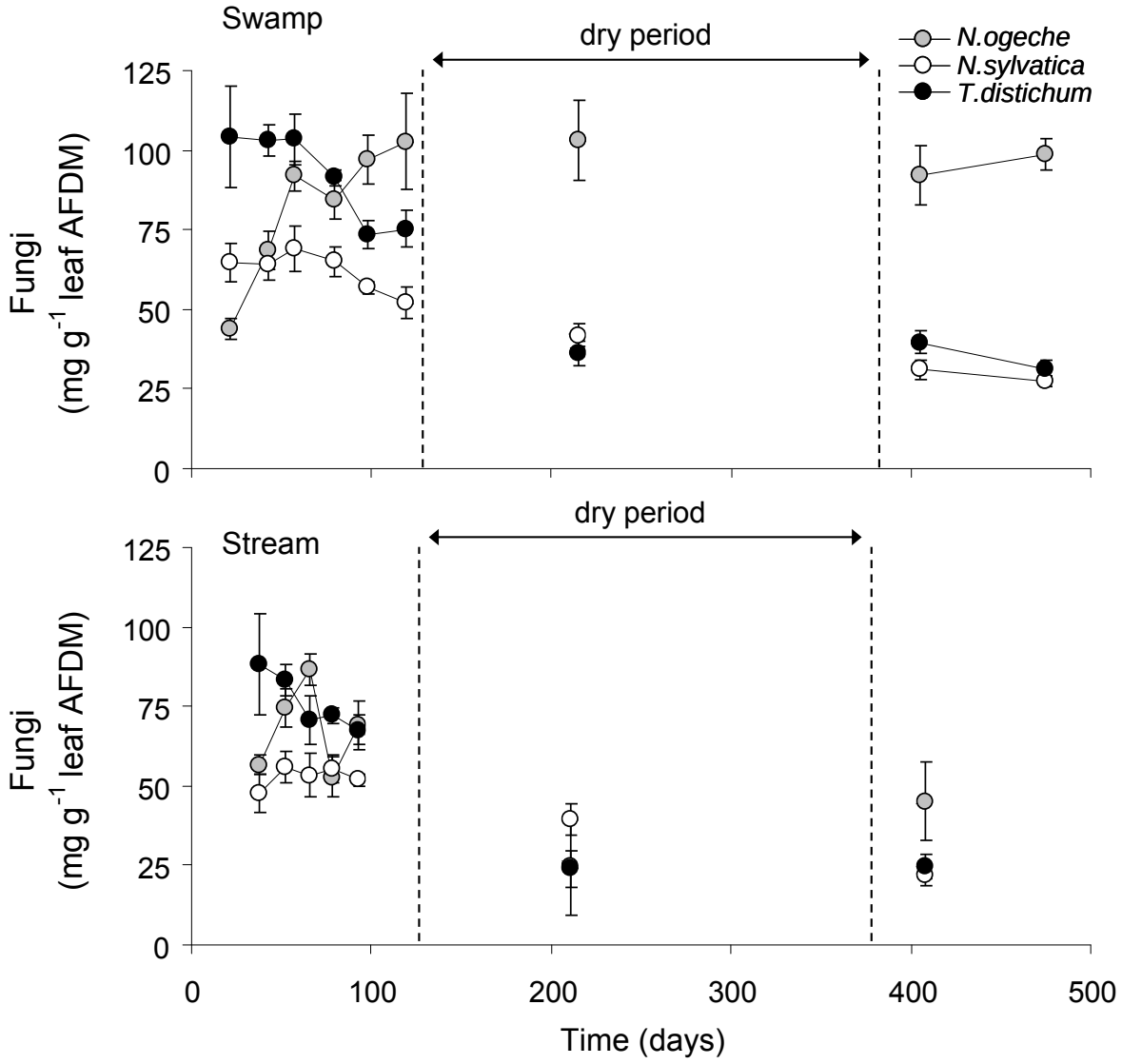

Swamp
dry period
N.ogeche
N.sylvatica
T.distichum
Fungi
(mg g-1 leaf AFDM)
125
100
75
50
25
0
Stream
dry period
Fungi
(mg g-1 leaf AFDM)
125
100
75
50
25
0
0
100
200
300
400
500
Time (days)

Figure 3.4: Leaf litter bacterial biomass over time in the swamp and stream. Dashed vertical lines indicate the beginning and end of the dry period.

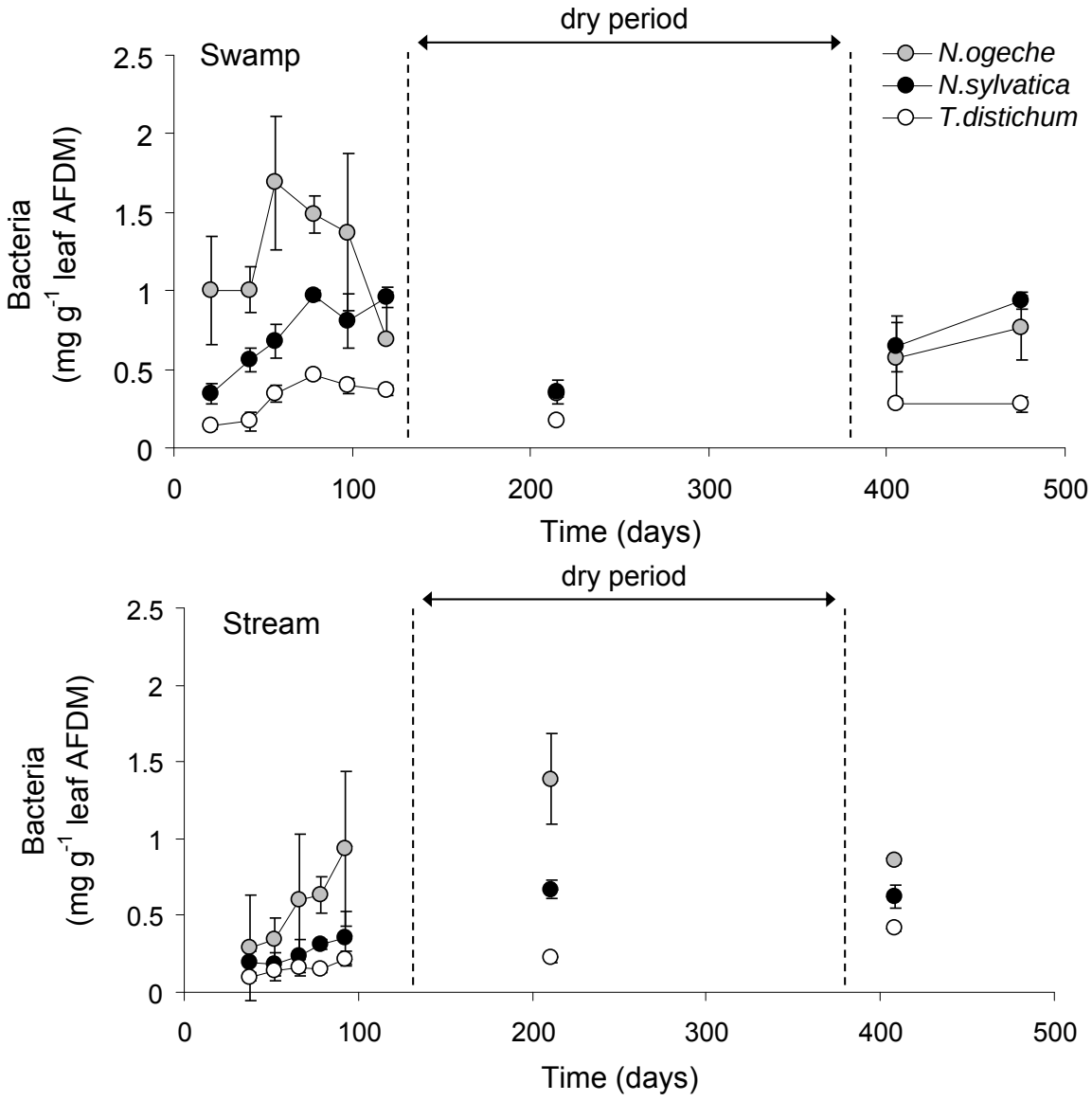

dry period
Swamp
N.ogeche
N.sylvatica
T.distichum
Bacteria
(mg g-1 leaf AFDM)
Time (days)
dry period
Stream
Bacteria
(mg g-1 leaf AFDM)
Time (days)

Figure 3.5: Shredder biomass over time in the swamp and stream, expressed per gram of leaf litter (top panels) and per leaf pack (bottom panels). The vertical dashed line indicates the break between wet periods.

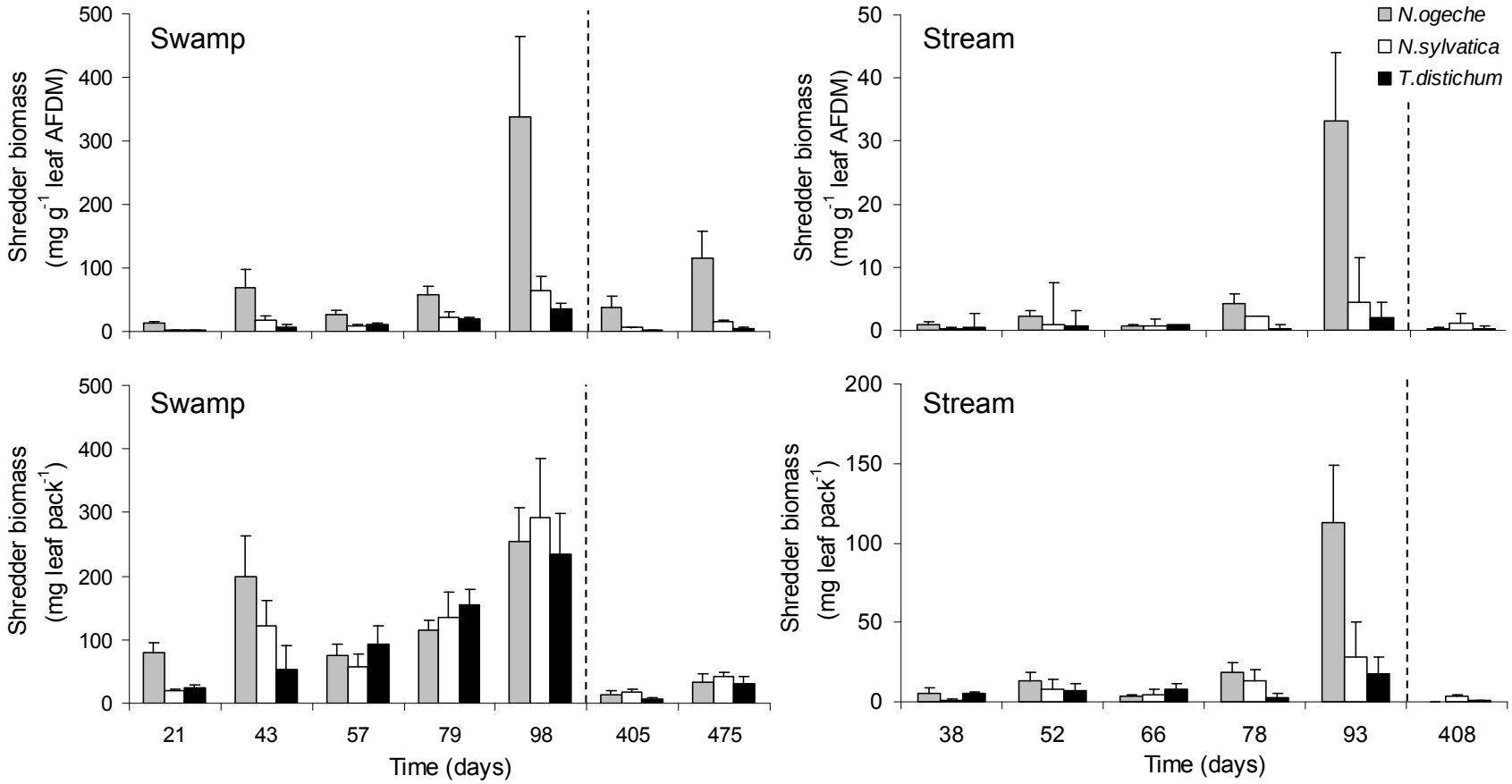

Swamp
Stream
N.ogeche
N.sylvatica
T.distichum
Shredder biomass (mg g-1 leaf AFDM)
Shredder biomass (mg leaf pack-1)
Time (days)
21
43
57
79
98
405
475
38
52
66
78
93
408

Figure 3.6: Breakdown rates (g day^{-1}) of leaf litter structural compounds per litter species in the swamp and stream. Different letters indicate significant differences among means (Bonferroni correction).

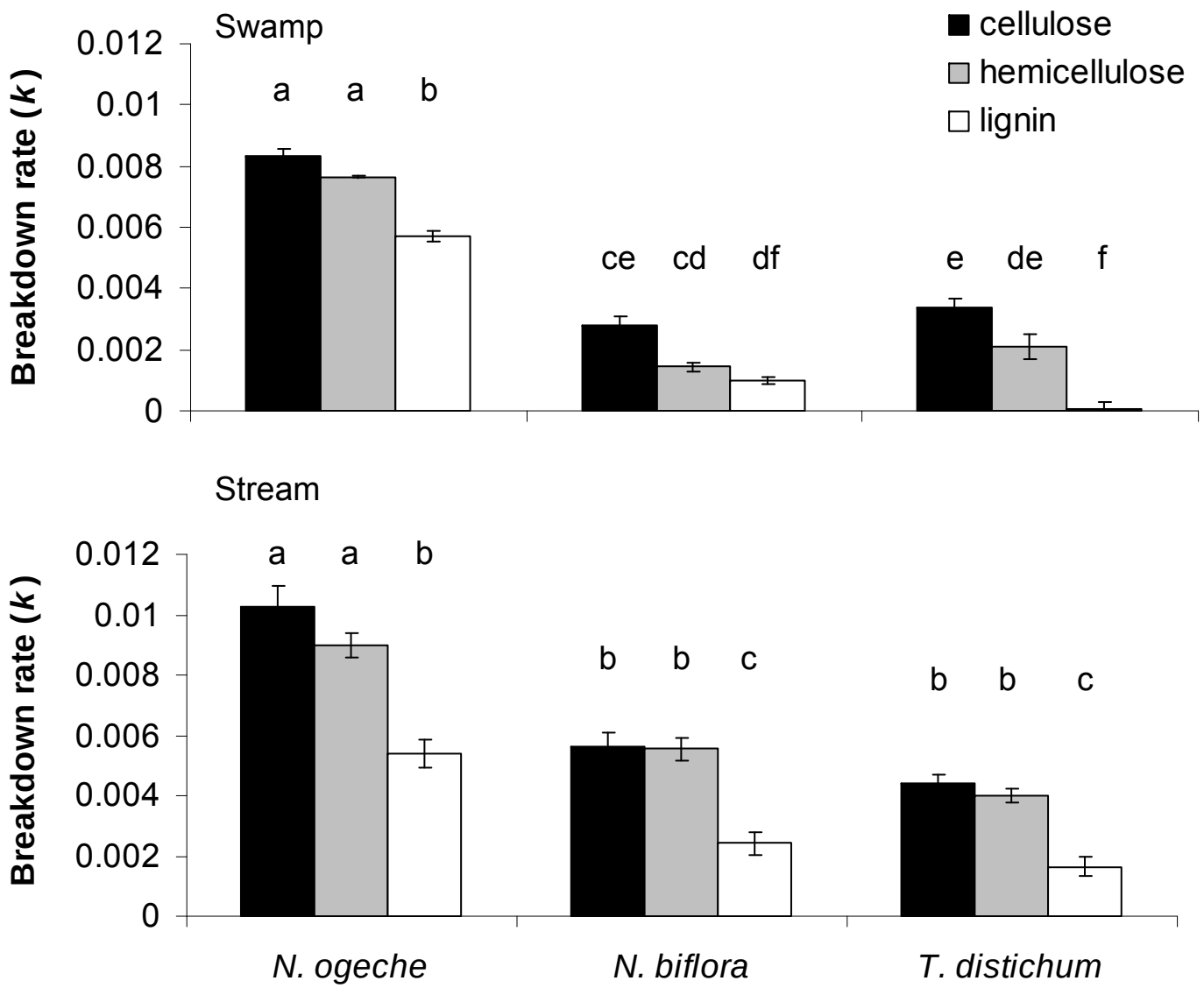
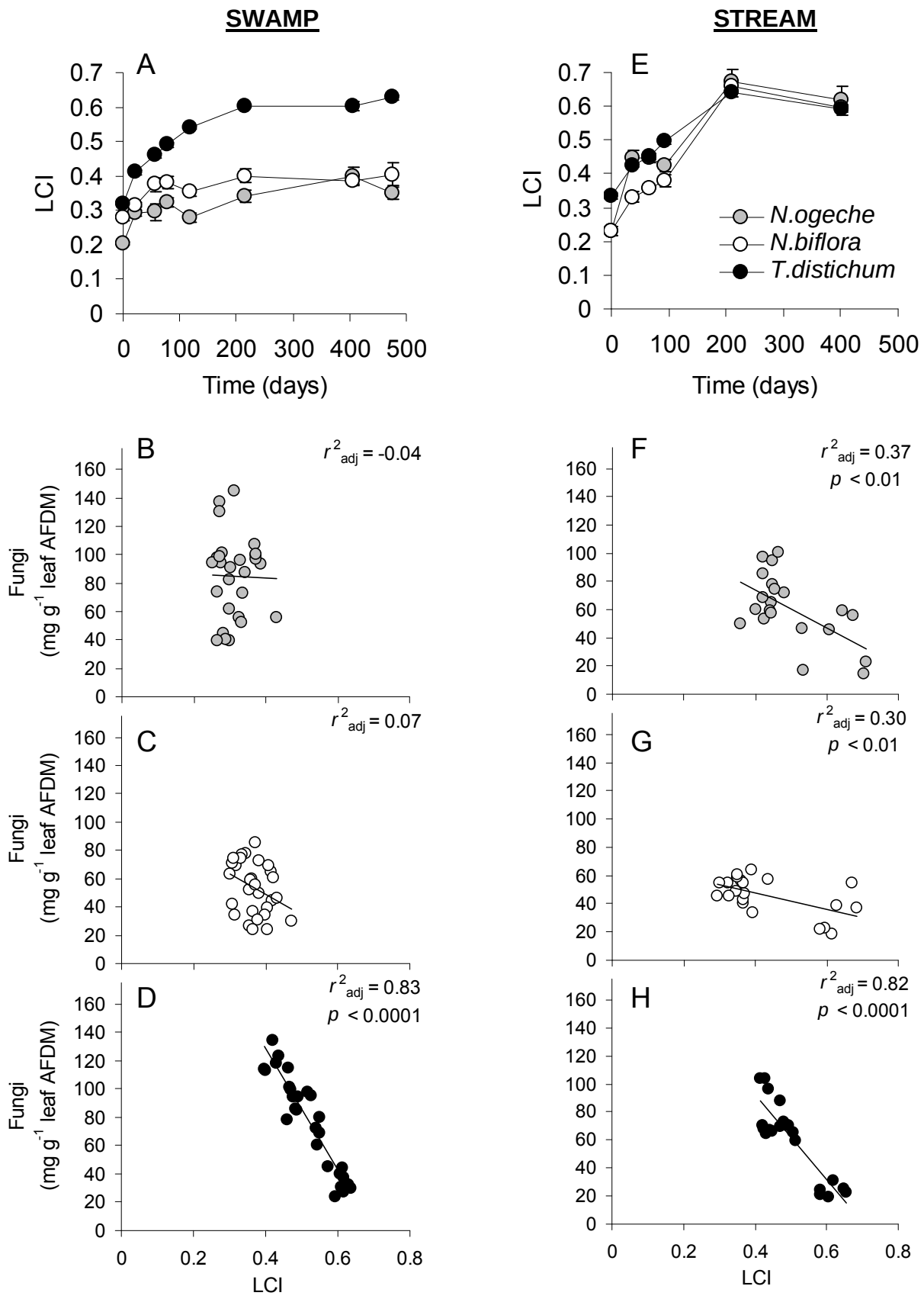

Swamp
Stream
cellulose
hemicellulose
lignin
Breakdown rate (k)
0.012
0.01
0.008
0.006
0.004
0.002
0
a a b
ce cd df
e de f
a a b
b b c
b b c
N. ogeche
N. biflora
T. distichum

Figure 3.7: Lignocellulose index (LCI) over time for *N. ogeche*, *N. biflora*, and *T. distichum* litter in the swamp (A) and stream (E), and regressed against fungal biomass in the three leaf litter species in the swamp (B-D) and stream (F-H).


SWAMP
STREAM
A
E
LCI
Time (days)
N.ogeche
N.biflora
T.distichum
B
C
D
F
G
H
Fungi (mg g-1 leaf AFDM)
r2adj = -0.04
r2adj = 0.07
r2adj = 0.83
p < 0.0001
r2adj = 0.37
p < 0.01
r2adj = 0.30
p < 0.01
r2adj = 0.82
p < 0.0001

CHAPTER 4

LEAF LITTER EFFECTS ON SEDIMENT OXYGEN DEMAND IN BLACKWATER RIVERS: THE ROLES OF FOREST COMPOSITION AND HYDROLOGY[1]

[1]Andrew S. Mehring, George Vellidis, Kevin A. Kuehn, Catherine M. Pringle, Cynthia J. Tant and R. Richard Lowrance. To be submitted to *Freshwater Biology*.

Abstract

Many blackwater streams and rivers of North America exhibit seasonally low dissolved oxygen, potentially stemming from microbial oxygen uptake during leaf litter breakdown. Here estimates of benthic leaf litter respiration are compared to direct measurements of sediment oxygen demand (SOD) in third- and fifth-order reaches (stream and swamp, respectively) of a blackwater river in Georgia's coastal plain. Leaf litter inputs, standing stocks, and litter-associated microbial biomass and respiration were measured directly during two wet seasons. Total annual litter inputs to the stream averaged 677 g m^{-2} y^{-1}, and leaf litter inputs were dominated by oak (231 g m^{-2}, 45%) and swamp tupelo (111 g m^{-2}, 21%), while total annual litter inputs to the swamp averaged 995 g m^{-2} y^{-1}, and were dominated by pond cypress (673 g m^{-2}, 74%), Ogeechee tupelo (80 g m^{-2}, 9%), and swamp tupelo (62 g m^{-2}, 7%). Detrital standing stock in both sites consisted primarily of leaf litter, accounting for ca. 65% of total. Leaf litter standing stocks changed significantly at both sites, with greater decreases observed in the stream (-609.83 g m^{-2}) versus the swamp (-493.69 g m^{-2}) during the 2008 wet season. A greater percentage of leaf litter inputs were retained in the swamp than in the stream (54% vs. 30%) after the wet period. In the swamp, mean areal estimates of microbial respiration from leaf litter (5.54 g O_2 m^{-2} day^{-1}) accounted for 89% of mean total SOD (6.20 g O_2 m^{-2} day^{-1}). However, SOD estimates ranged from 2.11 – 14.19 g O_2 m^{-2} day^{-1}, while leaf litter standing stock respiration estimates ranged from 2.07 – 8.00 g O_2 m^{-2} day^{-1}. Higher SOD values could not be accounted for by leaf litter or woody debris oxygen demand, suggesting that an additional oxygen sink occasionally contributes to SOD. Fungal biomass was significantly correlated to leaf litter oxygen uptake rates in the stream, but not in the swamp, potentially due to the effects of low oxygen on ergosterol-biomass ratios. Our findings demonstrate that leaf litter plays an important role in total

SOD within blackwater swamps, but additional drivers of SOD other than leaf litter and woody debris may be important as well. Leaf litter represents a substantial source of oxygen demand in these systems, and may be a large contributor to hypoxia especially in swamps, where low water velocity results in high retentiveness and reduced export of particulate organic matter.

Introduction

Oxygen availability is a critical factor determining the structure and function of aquatic ecosystems. Areas of hypoxia or anoxia may exist in freshwater ecosystems that are oxygenated throughout much of the water column, such as in the bottom waters of stratified lakes (Wetzel 2001), or in hyporheic zones of streams and rivers (Malard and Hervant 1999). Hypoxia throughout the water column of streams and rivers occurs less frequently, outside of cases involving anthropogenic nutrient loading (Mallin et al. 2006). However, many lotic ecosystems experience system-wide hypoxia for extended periods each year, apparently in the absence significant nutrient loading. Large wetlands such as the Pantanal (Hamilton et al. 1997), many estuaries (Pomeroy and Cai 2006), forested temporary ponds (Rubbo et al. 2006), and blackwater rivers (Meyer 1992) are aquatic ecosystems that may be "naturally" low in oxygen at predictable times of year. For example, blackwater rivers experience high temperatures, low discharge, and low dissolved oxygen (DO) during summer months (Mulholland 1981, Meyer 1992). Many of the blackwater rivers in North America's southeastern coastal plain exist in areas dominated by agriculture and silviculture (Brown et al. 2005), and although eutrophication can cause hypoxia in these systems (McCormick and Laing 2003, Mallin et al. 2004), many that are low in oxygen do not show obvious signs of nutrient loading (Meyer 1992, Carey et al. 2007, Todd et al. 2009).

Large stocks of dissolved and particulate organic carbon are characteristic of many blackwater rivers (Meyer et al. 1997, Smock 1997, Rixen et al. 2008), which may have the potential to increase DO demand. Decomposing organic matter can be a major sink for oxygen in aquatic ecosystems, and benthic oxygen demand is often correlated with the amount of sediment organic matter (Fuss and Smock 1996, Todd et al. 2009). Leaf litter often makes up the majority of organic matter inputs in forested streams (Cummins 1974, Vannote et al. 1980, Tank et al. 2010). Although total standing stocks of wood may be higher than leaf litter in blackwater rivers (Meyer et al. 1997, Smock 1997), yearly inputs of leaves outweigh those of wood (Mulholland 1981, Cuffney 1988). Leaf litter also has a higher turnover rate and supports higher microbial respiration rates than wood (Tank et al. 1993, Smock 1997, Webster et al. 1999, Stelzer et al. 2003). As a consequence, the degree of riparian leaf litter inputs and retention may potentially explain underlying patterns in DO dynamics observed in these systems.

A collaborative project between the University of Georgia (UGA) and the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) titled "Dissolved Oxygen Dynamics in the Upper Suwannee River Basin" was initiated to examine the factors affecting DO concentrations in the blackwater rivers of Georgia's coastal plain. In a previous study, Todd et al. (2009) examined sediment oxygen demand (SOD) within 5th-order reaches of the Little River. Observed SOD rates were among some of the highest reported for blackwater rivers, and although a positive correlation was observed between organic carbon concentration and SOD, the various sources and contribution of the organic carbon substrates (i.e. leaf litter, wood, algae) to oxygen demand were not determined.

In a 3rd-order (stream) and 5th-order (swamp) reach of the Little River, our objectives are to 1) determine leaf litter standing stock contributions to benthic oxygen demand, 2)

determine the biotic and abiotic factors affecting litter inputs and changes in litter standing stock throughout the wet season, and 3) determine the relative contributions among leaf litter species and leaf-litter-associated microorganism. We predict that 1) leaf litter respiration per area will not be significantly different from SOD measured directly by Todd et al. (2009), 2) differences in forest composition and hydrology will determine differences in litter inputs and standing stock, and 3) oxygen uptake will differ among leaf litter species and will be correlated to fungal biomass.

Methods

Study sites – This study was conducted in heavily forested third- and fifth-order reaches of the Little River, a blackwater river located in Georgia's coastal plain. The two reaches flow through Turner and Tift counties, respectively, and are contained within the Little River Experimental Watershed (LREW). Located in the headwaters of the upper Suwannee River basin, the 33,400-ha watershed was instrumented beginning in 1967 for rainfall and stream flow measurements by the USDA-ARS Southeast Watershed Research Laboratory (SEWRL) and includes eight nested gauged sub-watersheds ranging in size from 260 to 11,500 ha. Detailed records of stream flow, nutrient concentrations, and DO concentrations are regularly collected in each sub-watershed.

The third-order reach (31º41'32"N, 83º42'09"W, hereafter referred to as "stream") drains a 2,200 ha catchment, and meanders through a second-growth forest floodplain with moderate water velocity. Low-oxygen events occur during spring and summer, but oxygen concentrations rarely approach zero, and infrequently drop below 4 ppm. The fifth-order reach (31º28'54"N, 83º,35'03"W, hereafter referred to as "swamp") drains the entire 33,400 ha catchment, and

contains a large swamp where the channel widens to roughly 300 meters, and water velocity is substantially lower than in the stream. Low oxygen events are more frequent in the swamp, and oxygen concentrations are generally below 1 ppm from early April until flow stops. The swamp reach is bordered on the east primarily by sandhills and pine forest, and on the west by low-intensity livestock operations with intact forested riparian buffer strips. Both reaches are intermittent, and dry completely during summer and fall. Summary chemical and physical variables are shown in Table 1, and temporal changes in discharge and DO concentrations are shown in Figure 4.1.

Litterfall sampling – In the swamp, litterfall collectors were installed every 25 m along five transects spaced roughly 150 m apart. In the stream, five transects were spaced roughly 70 m apart, and one litterfall collector was installed at the point where each transect crossed the center of the stream channel. Additional collectors were located five meters to either side of the central point, and all other collectors were spaced ten meters apart. Transects reached to the farthest extent of the wetted perimeter of the floodplain during high discharge events. Litterfall collectors were constructed from round plastic laundry baskets equipped with drain holes and lined with 1-mm mesh. Baskets were mounted on metal poles 1 meter from the ground (Fig. 4.2) surface to prevent submergence during high flows. Leaf litter was collected monthly, except during periods of heavy leaf fall, when litter were collected bi-weekly. There were a total of 49 litterfall collectors in the swamp and 56 in the stream and its associated floodplain. Collection in both sites began on October 1, 2006. The point-centered quarter method (Cottam and Curtis 1956) was used at each litterfall sampling point within a site to estimate forest composition at each site. Diameter at breast height (dbh, 1.2 meters) and distance to the nearest tree > 2.5-cm dbh in the

northeast, northwest, southeast, and southwest quarters were measured, and each tree was identified to species.

Standing stock – Standing stocks of leaf litter and small woody debris (≤ 5 cm diameter), and leaf-associated fungal biomass? were estimated in February, March, and December 2007, and in April and August 2008 according to methods described by (Suberkropp et al. 2010) and (Pozo and Elosegi 2005). An interval was established upstream of each litterfall collection transect. In the swamp, ten random transects were spaced ten meters apart within each of the five intervals. In the stream, five random transects were spaced ten meters apart within each interval. Standing stock was collected at points ¼ , ½, and ¾ across the main channel, with distances along transects estimated using a map of the channel.

The average number of paces required to walk a given distance (0.67 meters per stride) across the swamp was measured during the dry season, and on each sampling date distances across the channel were estimated by counting paces. Once the sampling location was reached, a modified surber sampler (0.45-m-diameter square PVC frame with a 500 µm mesh drift net) was used to collect all CPOM (except for wood > 5 cm diameter) from the stream bed surface. Immediately upstream of the PVC frame, a 1-mm-mesh barrier mounted on metal rebar prevented material from drifting into the frame. All material within the PVC frame was collected no deeper than 1 cm below the water-sediment interface, following methods described by (Suberkropp et al. 2010). Standing stock samples were placed into clean, individually-labeled resealable plastic bags filled with stream water, and immediately placed on ice and transported to the laboratory for further processing.

Microbial respiration and biomass – In situ rates of microbial respiration from the most common leaf litter species were measured at one randomly-selected point per transect (five

points per date), using methods described by Suberkropp et al. (2010). Briefly, ten 17-mm-diameter disks were cut from a single species and immediately enclosed into a 26-mL respiration chamber containing unfiltered stream water. Changes in DO concentrations within chambers were measured every 5 minutes for 30 minutes using a YSI 5100 Dissolved Oxygen Meter (Yellow Springs, OH, U.S.A.). All measurements were conducted at ambient stream water temperatures in darkness. Oxygen uptake rate was determined by the slope of the regression of DO concentrations versus time, minus a control slope measured using stream water alone. Following respiration measurements, leaf discs were placed into labeled foil packets, placed on ice, and transported to the laboratory. Leaf litter samples from the most common leaf litter species were collected at each of the fifteen sample points on each date for estimating litter associated fungal biomass (ergosterol). Five 12-mm-diameter disks were cut from collected litter material and placed into clean 20-ml plastic scintillation vials and preserved with 5 ml HPLC-grade methanol. All samples were immediately placed on ice and transported to the laboratory where they were stored in the dark at -20ºC (fungal biomass) until analysis.

Standing stock litter processing – Upon returning to the lab, standing stock litter samples were immediately frozen at -20˚C until later processing. During processing, litter was rinsed over a sieve (1 mm mesh size) to remove fine particulates (< 1 mm in size), and sorted by species and type (wood, leaves, fruit, flowers, etc.). Sorted samples were dried for one week at 60ºC, weighed, and random sub-samples were combusted at 500ºC to determine percentage ash-free dry mass (AFDM) and to correct for accumulated inorganic sediments. Litterfall samples and standing stock collected during dry periods were not rinsed, but in all other aspects were processed as described above.

Fungal biomass – Fungal biomass was estimated from ergosterol concentrations in preserved leaf litter samples. Ergosterol was extracted in alcoholic KOH (0.8% KOH in methanol, total extraction volume 10 ml) for 30 minutes at 80ºC in tightly capped tubes with constant stirring. The resultant crude extract was partially cleaned by solid phase extraction (Gessner and Schmitt 1996), and ergosterol quantified by high-pressure liquid chromatography (HPLC). A LichroSpher 100 RP-18 column (0.46 × 25 cm, Merck) maintained at 40°C in a Shimadzu column oven (CTO-10AS) and connected to a Shimadzu autosampler (SIL-10AD) and Shimadzu liquid chromatograph system (Pumps LC-10AT, Controller SCL-10A) was used for separation and analysis. The mobile phase was HPLC grade methanol at a flow rate of 1.5 ml min^{-1}. Ergosterol was detected at 282 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time = ca. 8 min), and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical).

Results

Forest composition – In the stream, the dominant tree species differed depending on whether the floodplain and higher elevations were included in the analysis (Fig. 4.3A). Dominant species (in order of decreasing importance values) were oak, swamp tupelo, red maple, and Ogeechee tupelo across the floodplain, while swamp tupelo, red maple, oak, and Ogeechee tupelo dominated the main channel. The swamp was dominated by pond cypress, swamp tupelo, Ogeechee tupelo, and red maple (Fig. 4.3B).

Litterfall – Rates of litterfall differed seasonally in the stream ($F_{11,48} = 12.62$, $p < 0.0001$, Fig. 4A) and swamp ($F_{11,47} = 38.29$, $p < 0.0001$, Fig. 4.4B), with highest rates occurring during Fall months in both sites. The abscission of pond cypress pollen cones during March and early

April resulted in a second pulse of litter input, with rates significantly higher than those observed in February, June and July (Bonferroni, all $p < 0.005$, Fig. 4.4B). The composition and amount of leaf litter inputs from tree species differed from the species of highest importance in forest composition surveys. Total annual litter inputs to the stream averaged 677 g m^{-2} y^{-1}, which were dominated by oak (231 g m^{-2}, 45%), swamp tupelo (111 g m^{-2}, 21%), pine (69 g m^{-2}, 13%), red maple (63 g m^{-2}, 12%) and Ogeechee tupelo (42 g m^{-2}, 8%). Total annual litter inputs to the swamp averaged 942 g m^{-2} y^{-1}, which were dominated by pond cypress (673 g m^{-2}, 74%), Ogeechee tupelo (80 g m^{-2}, 9%), swamp tupelo (62 g m^{-2}, 7%). In the swamp, pond cypress litter inputs per total trunk biomass per area (g dbh^{-1} m^{-2} y^{-1}) were 42.99 ± 23.14 (± 1 S.E.) in shallow areas, 139.69 ± 42.05 in areas of moderate depth, and 174.08 ± 35.91 in the deepest portions of the swamp, and were significantly higher than those of swamp tupelo and Ogeechee tupelo ($F_{2,141} = 4.61$, $p < 0.05$, Fig. 4.5A). This was unrelated to pond cypress tree density within 5-meter radii around each survey point ($F_{1,31} = 0.02$, $p > .90$), and pond cypress was only significantly more dominant in the deepest portions of the swamp ($F_{2,42} = 20.09$, $p < 0.001$, Fig. 4.5B).

Standing stock – Detritus standing stock in both sites consisted primarily of leaf litter (~ 65% of total in December 2007, Fig. 4.6A). In the stream, leaf litter standing stocks changed significantly during the wet season ($F_{2,18} = 5.50$, $p < 0.05$, Fig. 4.6A), decreasing from 867.32 ± 227.33 (± 1 S.E.) to 112.59 ± 43.50 g m^{-2} between mid-December and early April (Tukey, $p < 0.05$). During the dry season, leaf litter standing stocks were slightly higher in August than in April in the stream, but the difference was not statistically significant (Tukey, $p > 0.12$) and was partially due to freshly-abscised leaf litter. In the swamp, leaf litter standing stocks changed significantly over time during 2007 and 2008 ($F_{4,32} = 12.05$, $p < 0.0001$, Fig. 4.6B). Leaf litter

standing stocks in the swamp decreased from 629.16 ± 77.41 to 470.70 ± 47.57 g m^{-2} between mid-February and late March 2007, but the change was not statistically significant (Tukey, p > 0.45, Fig. 4.6B). Largest decreases occurred between mid-December 2007 and early April 2008, from 1080.40 ± 76.68 (± 1 S.E.) to 673.69 ± 55.66 g m^{-2} (Tukey, $p < 0.001$). Leaf litter standing stocks decreased further to 586.71 g m^{-2} by August 2008, but the difference was not statistically significant (Tukey, $p > 0.87$). Wood and other non-leaf litter standing stocks did not change significantly over time at either site. Decreases in leaf litter standing stock between December 2007 and August 2008 were greater in the stream (-609.83 g m^{-2}) than in the swamp (-493.69 g m^{-2}). A percentage of the leaf litter standing stocks initially present in December 2007 remained in the stream (30%) and swamp (54%) channel in August 2008, after the transition from wet to dry phases.

Oxygen demand – In the stream, areal respiration rates from decaying leaf litter averaged 0.88 ± 0.35 g O_2 m^{-2} day^{-1} in April 2008 (range: 0.01-2.66). These were significantly lower than total SOD measurements taken directly in February 2007 ($F_{1,13} = 7.46$, $p < 0.02$, Fig. 4.7A), which averaged 2.36 ± 0.68 g O_2 m^{-2} day^{-1} (range: 0.49-4.62). In the swamp, leaf litter standing stock respiration rates changed significantly over time ($F_{2,18} = 7.34$, $p < 0.005$, Fig. 4.7B), and averaged 3.65 ± 0.46, 5.69 ± 0.54, and 7.29 ± 0.59 g O_2 m^{-2} day^{-1} in February and March 2007, and April 2008, respectively. Across all dates, the mean areal respiration rate (5.54 g O_2 m^{-2} day^{-1}) accounted for 89% of mean total SOD (6.20 g O_2 m^{-2} day^{-1}). The two estimates were not significantly different ($F_{1,36} = 1.34$, $p > 0.59$, Fig. 4.6B), and temperature had no significant effect across the two estimation methods and all sampling dates ($F_{1,36} = 2.58$, $p < 0.11$), confirming findings of Todd et al (2010) that observed no effect of temperature on SOD estimates. Comparing temperature-corrected areal leaf litter respiration rates to SOD measured

two days apart (late March 2007) provides a more reasonable comparison. Mean temperature-corrected areal respiration rates from leaf litter (4.38 g O_2 m^{-2} day^{-1}) accounted for 71% of mean total SOD (6.16 g O_2 m^{-2} day^{-1}). Estimates provided by the two methods were not significantly different ($F_{1,9} = 0.89$, $p > 0.36$), but microbial respiration associated with leaf litter cannot account for all of the oxygen-consuming processes on the swamp basin. SOD estimates range from 2.11 – 14.19 g O_2 m^{-2} day^{-1}, while leaf litter standing stock respiration estimates range from 2.07 – 8.00 g O_2 m^{-2} day^{-1}. Although the low ranges are similar, higher SOD values are difficult to account for with leaf litter oxygen demand alone.

Fungal biomass and temperature effects on oxygen uptake – Fungal standing crop (mg m^{-2}) was higher in the swamp (Fig. 4.8A), due to higher standing stocks of litter and also to slightly higher fungal biomass per gram of leaf tissue in pond cypress litter (Fig. 4.8B, C). In the stream, fungal biomass differed significantly among litter species ($F_{3,31} = 27.89$, $p < 0.0001$), with fungal biomass associated with *Pinus* sp. litter being significantly lower than on all other litter species (Tukey, all $p < 0.001$). Fungal biomass concentrations associated with red maple litter were significantly higher than those observed on water oak and *Pinus* sp. litter (Tukey, all $p < 0.01$). Leaf litter oxygen uptake also differed among litter species ($F_{3,14} = 4.11$, $p < 0.05$) and was significantly influenced by the amount of fungal biomass ($t_{1,15} = 3.26$, $p < 0.01$, $r^2_{adj} = 0.45$, Fig. 4.8B). In the swamp, there was a significant interaction effect of tree species and time on fungal biomass ($F_{2,74} = 7.40$, $p < 0.01$). Fungal biomass in swamp tupelo litter did not change significantly over time, but fungal biomass in pond cypress decreased significantly between mid-February and late March 2007 (Tukey, $p < 0.05$). Pond cypress supported significantly higher fungal biomass than swamp tupelo litter in mid-February and late March 2007 (Tukey, all $p < 0.01$), but differences between species were not significant in April 2008. Oxygen uptake per

gram of leaf changed over time ($F_{2,17} = 6.34$, $p < 0.01$) but did not differ significantly between swamp tupelo and pond cypress across sampling dates ($F_{1,17} < 0.01$, $p > 0.99$). Most of the variation over time was explained by differences in temperature ($t_{1,23} = 4.52$, $p < 0.001$, $r^2_{adj} = 0.46$). Although a moderately significant relationship between leaf litter fungal biomass and O_2 uptake existed in the swamp in February 2007 ($t_{1,7} = 2.16$, $p = 0.067$, $r^2_{adj} = 0.31$, Fig. 4.8C), across all sampling dates the relationship between fungal biomass and oxygen uptake was not significant. Oxygen uptake rates were slightly higher in the stream than in the swamp. This may have been due to reduced oxygen availability while taking respiration measurements, or perhaps to physiological changes in fungi activity due to prolonged time in low-oxygen conditions. Fungal biomass concentrations of ergosterol, the indicator used here, have been demonstrated to vary considerably with changes in DO availability (Charcosset and Chauvet 2001).

Discussion

Ecosystem metabolism is influenced by the amount of allochthonous detritus entering a system, and also by how efficiently those materials are utilized by consumers or exported from the system. The swamp received more allochthonous inputs but also exported less (as a percentage of inputs and also as an absolute value of leaf litter) than the stream, likely due to much lower water velocity. The increased retentiveness of swamps combined with large leaf litter inputs by flood-adapted trees makes them potentially large sinks for DO.

Compared to other cypress-dominated swamps, litterfall within the Little River swamp site (995 g m^{-2} y^{-1}) was higher than average (693 g m^{-2} y^{-1}) and within the upper third of the range of published literature values (79-1426 g m^{-2} y^{-1}) (Middleton and McKee 2004). Within the swamp, pond cypress also produced more litter per trunk diameter per area than either swamp

tupelo or Ogeechee tupelo. This is likely due to conditions within the site favoring the growth and production of pond cypress: intermittent drying and flooding as opposed to permanent flooding (Megonigal et al. 1997), moderately elevated nutrient levels (Brown 1981), and low redox potential favoring elevated concentrations of metals toxic to other tree species (McLeod and Ciravolo 2003). Although pond cypress has a more open crown than bald cypress, their leaves are significantly thicker and heavier, they produce more branchlets, and have a higher leaf area ratio than bald cypress (Neufeld 1983, 1986). The prevalence of this variety of *T. distichum* in the swamp may explain the relatively high rates of litterfall per cypress trunk.

Temporal changes in litter standing stock differed between the stream and swamp, with greater decreases observed in the stream. This is counter to what was suggested by the results of previous litter breakdown studies in the two sites, in which pond cypress, swamp tupelo and Ogeechee tupelo litter decomposed more rapidly in the swamp (chapter 3). Consequently, higher water velocities may have been exporting larger quantities of leaf litter out of the stream reach. Maximum water velocity in the stream (1.61 m s^{-1}) was 7.43× higher than maximum water velocity in the swamp (0.22 m s^{-1}), thus implying a greater capacity for the transport of leaf litter and other particulate organic matter. Slow water velocity in the swamp could result not only in enhanced retention of leaf litter falling from the canopy above, but also in enhanced retention of particulate organic matter entering the swamp from less retentive upstream reaches. Determining the frequency of these large in-stream swamps throughout Georgia's coastal plain may help to explain differences among rivers, and reaches within a river, in the relative retentiveness and accumulation of particulate organic matter and resulting differences in SOD among reaches.

Slow water velocities also increase the residence time of water and duration of contact between water and leaf litter within a channel. Todd (2008) used multiple injections of

rhodamine dye to measure water travel times within the same stream and swamp reaches included in the current study, and observed substantially higher water residence times in the swamp. Assuming adequate mixing and a lack of sources of dissolved oxygen, the DO concentration in 500 liters of water could be reduced from 9.0 mg L^{-1} to 4.0 mg L^{-1} in 10.83 hours in the swamp, and in 2 days, 20 hours, and 11 minutes in the stream. Alternatively, assuming average water velocities of 0.22 and 0.03 m s^{-1} in the stream and swamp (as estimated for 2008 in the current study), the dissolved oxygen concentration of a 500-liter volume of water would be reduced from 9.0 mg L^{-1} to 4.0 mg L^{-1} after passing through 1.17 km of the swamp, or through 54 km of stream channel. Therefore, due both to higher rates of litterfall, greater retention of litter, and long water residence times, swamps may act as tremendous oxygen sinks in river networks. An understanding of their frequency throughout the coastal plain of Georgia would shed some light on their importance to water policy at landscape scales.

This study provides evidence that leaf litter breakdown and associated microbial activity are important drivers of SOD in the Little River. However, other contributors to SOD, such as wood breakdown, may also impact DO demand in these systems. Although the use of SOD chambers requires that larger pieces of wood are moved in order to make a seal between the chamber and the substrate, small wood was common in standing stock samples, often small enough to fit within the SOD chamber used by Todd et al. (2009). Previously, Tank et al. (1993) reported microbial respiration rates from decaying wood substrates in an Appalachian stream ranging from (0.24-0.72 mg O_2 g^{-1} wood AFDM d^{-1}). Applying their maximum value of oxygen uptake to our highest measurements of wood standing stock could add as much as -0.48 g O_2 m^{-2} d^{-1} to our estimates of oxygen demand. Another likely source of oxygen uptake in the swamp site is from decaying filamentous diatoms and algae. Late in the wet season, the diatom *Fragilaria*

becomes abundant on decaying floating wood, and if senescent filaments were to drift to the swamp bottom, they could contribute significantly to SOD (Butts and Evans 1978). Macroinvertebrate respiration could contribute to SOD, including abundant Chironomid midges and fingernail clams. Fingernail clams have been cited as an important contributor to SOD by Butts and Evans (1978), although in the Illinois streams that they surveyed clam densities were up to 18× higher than in the swamp in the Little River. Finally, methane provides a plausible and large source of oxygen demand (Watson et al. 1997), as it is released from lower soil layers. Release of methane may be enhanced during installation of SOD chambers. In the stream, an additional source of oxygen consumption may be found in dense networks of tree roots that were often encountered near the surface of the sediment,

In previous studies within the Little River (chapter 3), fungal and bacterial biomass were correlated to oxygen uptake in the stream, but not in the swamp. The same pattern has been observed here. As they make up roughly 99% of total microbial biomass in submerged leaf litter, fungi would be expected to make the largest contribution to oxygen uptake. A lack of correlation, however, may be due to other factors potentially affecting fungal organisms. Most notably, oxygen availability has been demonstrated to substantially change ergosterol concentration in fungal tissue (Charcosset and Chauvet 2001). Ergosterol is a reliable estimate of living fungal biomass, but it is not a direct measurement, and the ability to accurately estimate fungal biomass from ergosterol may be reduced in a system that is so often hypoxic. Further, as drastic shifts in inundation, temperature, and oxygen availability may also cause fungal community shifts, ergosterol per gram of fungal biomass would be expected to change as well (Gessner and Chauvet 1993). Fungal biomass per m^2 is roughly twice as high as that measured in

reference conditions in an Appalachian mountain headwater stream, and reflects the fact that, while fungal biomass is similar between the sites, standing stock of litter is significantly higher.

This river an exception to the River Continuum Concept (Vannote et al. 1980), as allochthonous inputs are actually higher in the 5th order swamp reach than in the 3rd-order stream reach. The Little River appears to be dominated by heterotrophic processes from the headwaters at least to 5th-order reaches, as has been observed in other Georgia blackwater rivers such as the Ogeechee (Meyer and Edwards 1990).

Previously, Cathey (2005) identified SOD and reaeration to be the two most sensitive parameters in the modeling of DO within the LREW, and required an SOD calibration value of 6.0 g O_2 m^{-2} day^{-1} for the validation of a DO model (DoSag). This was very close to the average SOD value of 6.20 g O_2 m^{-2} day^{-1}, measured years later in the swamp by Todd et al. (2009), and only slightly higher than the average value of leaf litter standing stock respiration (5.54 g O_2 m^{-2} day^{-1}) measured in this study. Our results and those from Todd et al. (2009) indicate high benthic oxygen uptake rates similar to the SOD calibration value reported by Cathey (2005). Given the high oxygen demand within the system, it is surprising that hypoxic conditions are not more common throughout the wet season. Unidentified sources of oxygen may exist within the Little River. Carey et al. (2007) demonstrated that periphyton growth in the Little River was primarily light limited and secondarily nutrient limited, but his measurements were made late in the season when a full canopy most likely enhanced light limitation. Closer examination of diel oxygen curves within the LREW show a clear signal of oxygen production during daylight hours, and an unidentified source of oxygen, large enough to counteract a significant proportion of demand in the system, likely exists.

Leaf litter breakdown represents a substantial source of oxygen demand that may explain a large proportion of the seasonal hypoxia observed in the Little River. Although this is a "natural" source of oxygen demand, it is one that is vulnerable to anthropogenic impacts, such as eutrophication, which could potentially enhance hypoxia. Nutrient loading significantly increases leaf-litter-associated microbial biomass and respiration (Gulis and Suberkropp 2003, Mallin et al. 2004, Suberkropp et al. 2010), potentially enhancing benthic oxygen demand. Furthermore, in the event of substantial water withdrawals due to agricultural water demand, retentiveness and leaf litter standing stocks will likely increase. Decreased water velocity and decreased moisture during dry periods may reduce physical export and breakdown of litter, and potentially higher litter production by the swamp forest in the event of reduced flooding could increase litter inputs. If benthic oxygen demand was enhanced by higher stocks of leaf litter, oxygen levels may fall even further, especially if coupled with higher temperatures.

Acknowledgements

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Table 4.1: Summary physical and chemical data for two study reaches within the Little River Experimental Watershed (LREW) during 2007-2008.

	Third-order reach (stream)	**Fifth-order reach (swamp)**
DOC (mg L^{-1})	11.25 ± 0.68	22.89 ± 1.47
SRP (µg L^{-1})	9.83 ± 4.09	22.67 ± 1.47
Total P (µg L^{-1})	46.50 ± 10.38	79.59 ± 28.38
NO_3^- (µg L^{-1})	27.27 ± 8.30	33.07 ± 10.58
NH_4^+ (µg L^{-1})	28.13 ± 7.27	36.54 ± 9.00
Total N (µg L^{-1})	1199.47 ± 255.13	969.63 ± 249.00
pH	5.76 ± 0.08	6.11 ± 0.04
Discharge (L s^{-1})	353.29 ± 40.34	3343.06 ± 445.66
Velocity (m s^{-1})	0.22 ± 0.02	0.032 ± 0.0036

Figure 4.1: Discharge and dissolved oxygen (DO) throughout 2007 in the A) stream site and B) the swamp site.

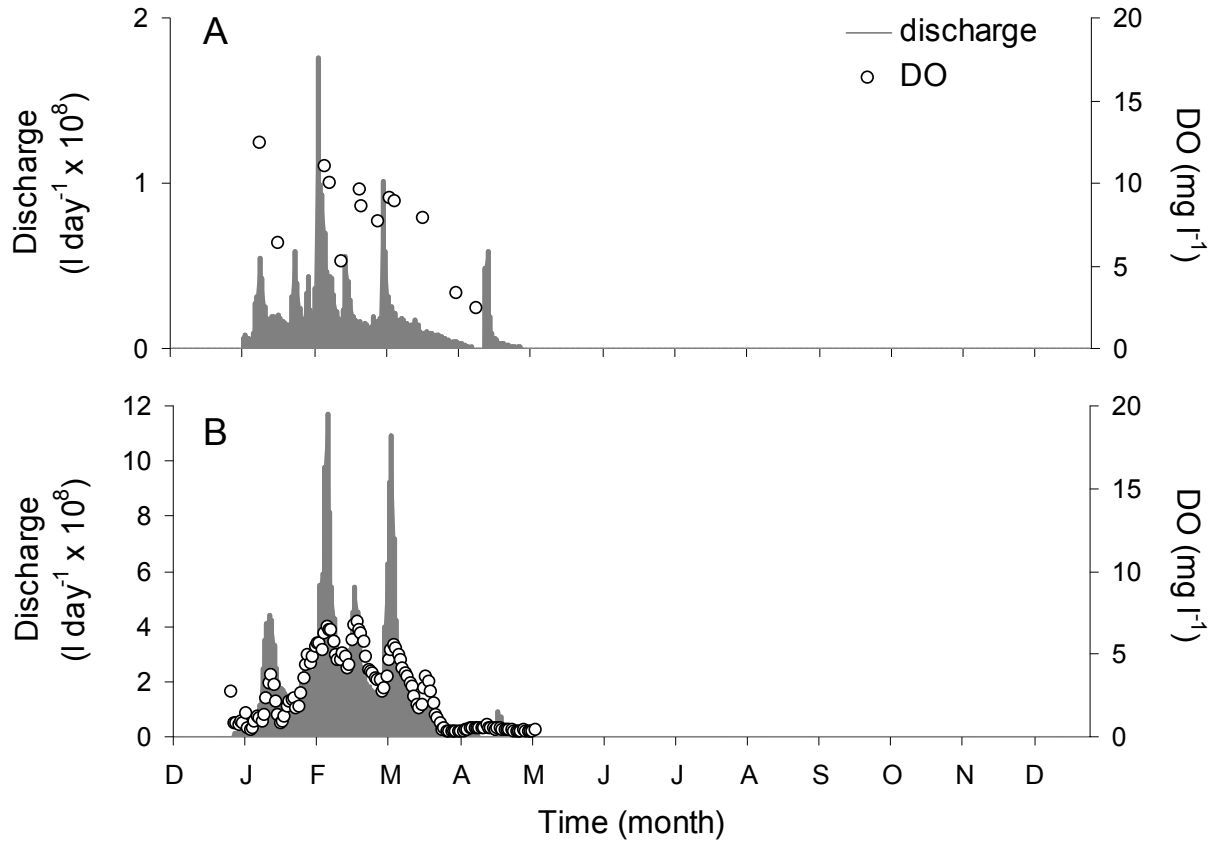
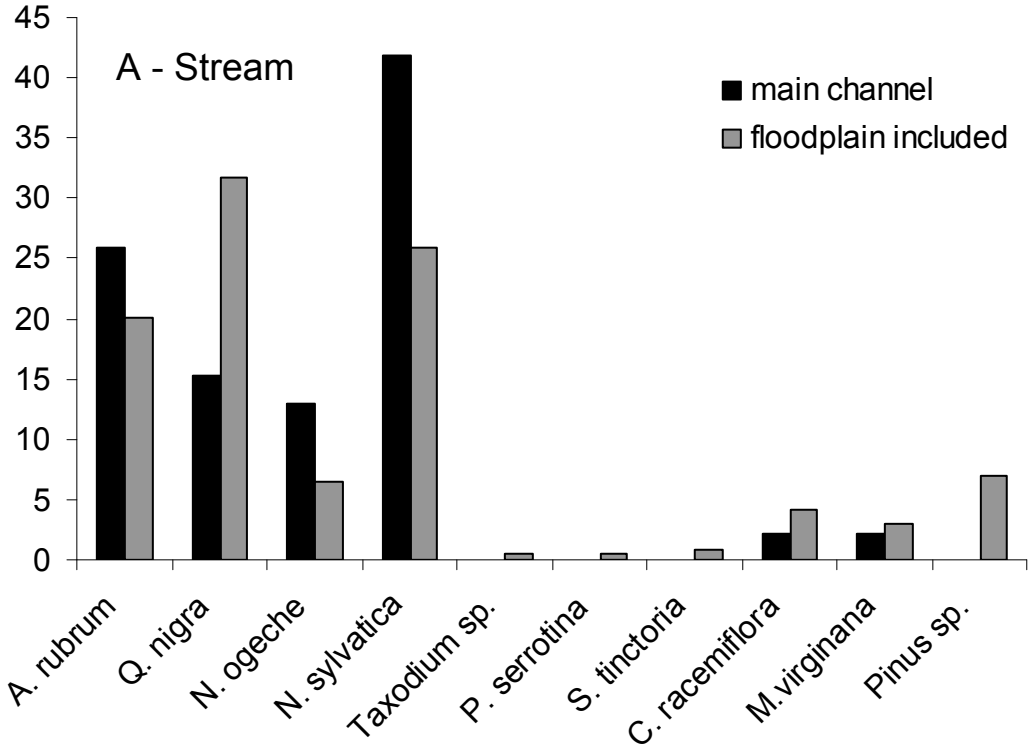

A
B
discharge
DO
Discharge (l day-1 x 10^8)
DO (mg l-1)
Time (month)
D
J
F
M
A
M
J
J
A
S
O
N
D

Figure 4.2: Litterfall collectors in the swamp during A) wet and B) dry periods.


A


B

Figure 4.3: Forest composition importance values in the A) stream site and B) swamp site. Estimates in the stream site are provided including and excluding the floodplain.


A - Stream
main channel
floodplain included
45
40
35
30
25
20
15
10
5
0
A. rubrum
Q. nigra
N. ogeche
N. sylvatica
Taxodium sp.
P. serrotina
S. tinctoria
C. raceminflora
M.virginana
Pinus sp.

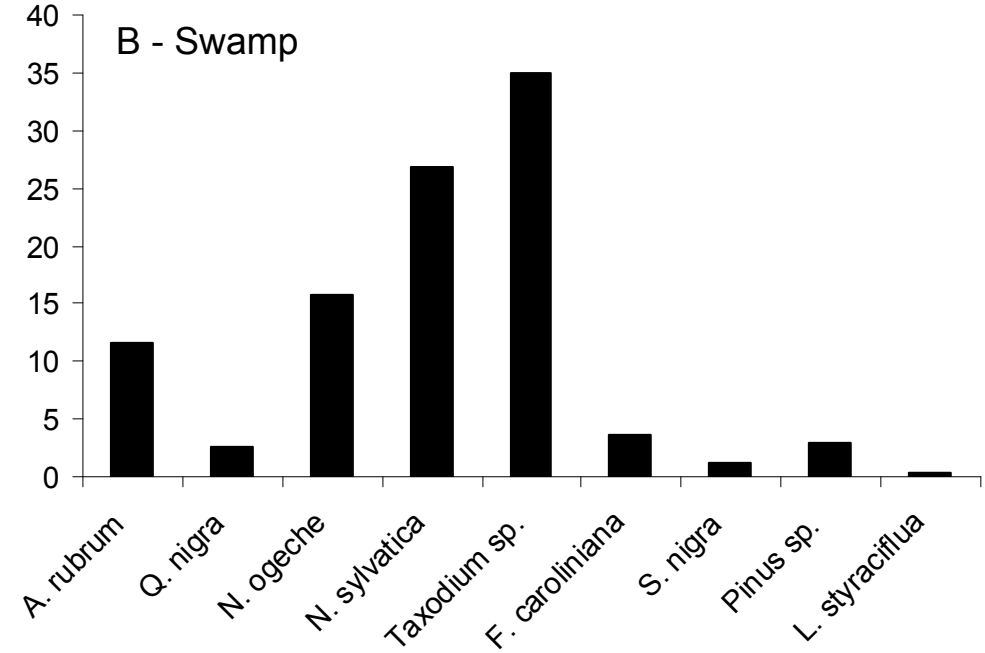

B - Swamp
40
35
30
25
20
15
10
5
0
A. rubrum
Q. nigra
N. ogeche
N. sylvatica
Taxodium sp.
F. caroliniana
S. nigra
Pinus sp.
L. styraciflua

Figure 4.4: Litter inputs for A) the main channel of the stream reach, and B) central sampling points along transects in the swamp.

Figure 4.4.

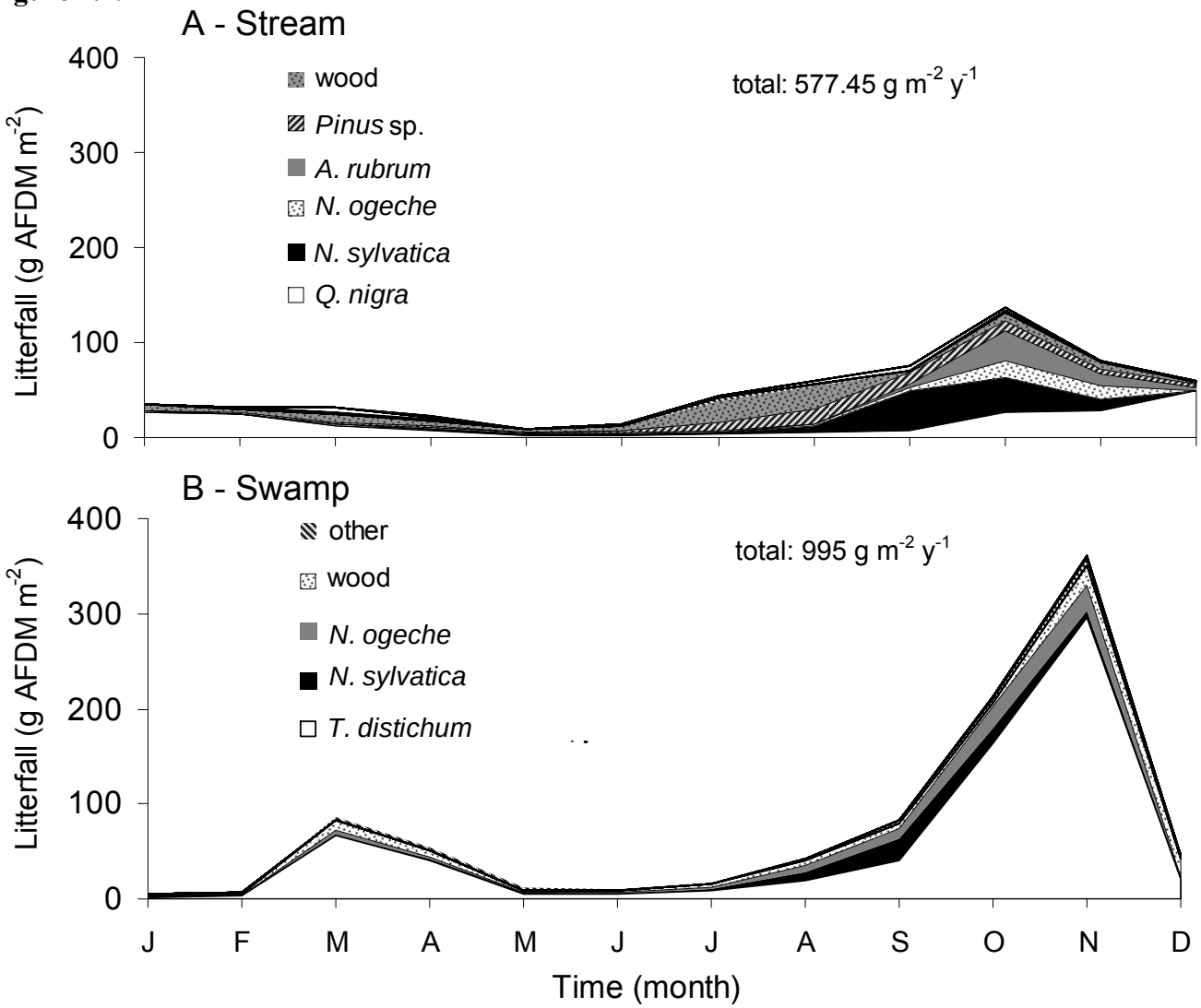

A - Stream
wood
Pinus sp.
A. rubrum
N. ogeche
N. sylvatica
Q. nigra
total: 577.45 g m-2 y-1
Litterfall (g AFDM m-2)
400
300
200
100
0
B - Swamp
other
wood
N. ogeche
N. sylvatica
T. distichum
total: 995 g m-2 y-1
Litterfall (g AFDM m-2)
400
300
200
100
0
J
F
M
A
M
J
J
A
S
O
N
D
Time (month)

Figure 4.5: A) Water depth and tree species effects on quantity of litterfall and B) water depth effects on relative dominance in the swamp.

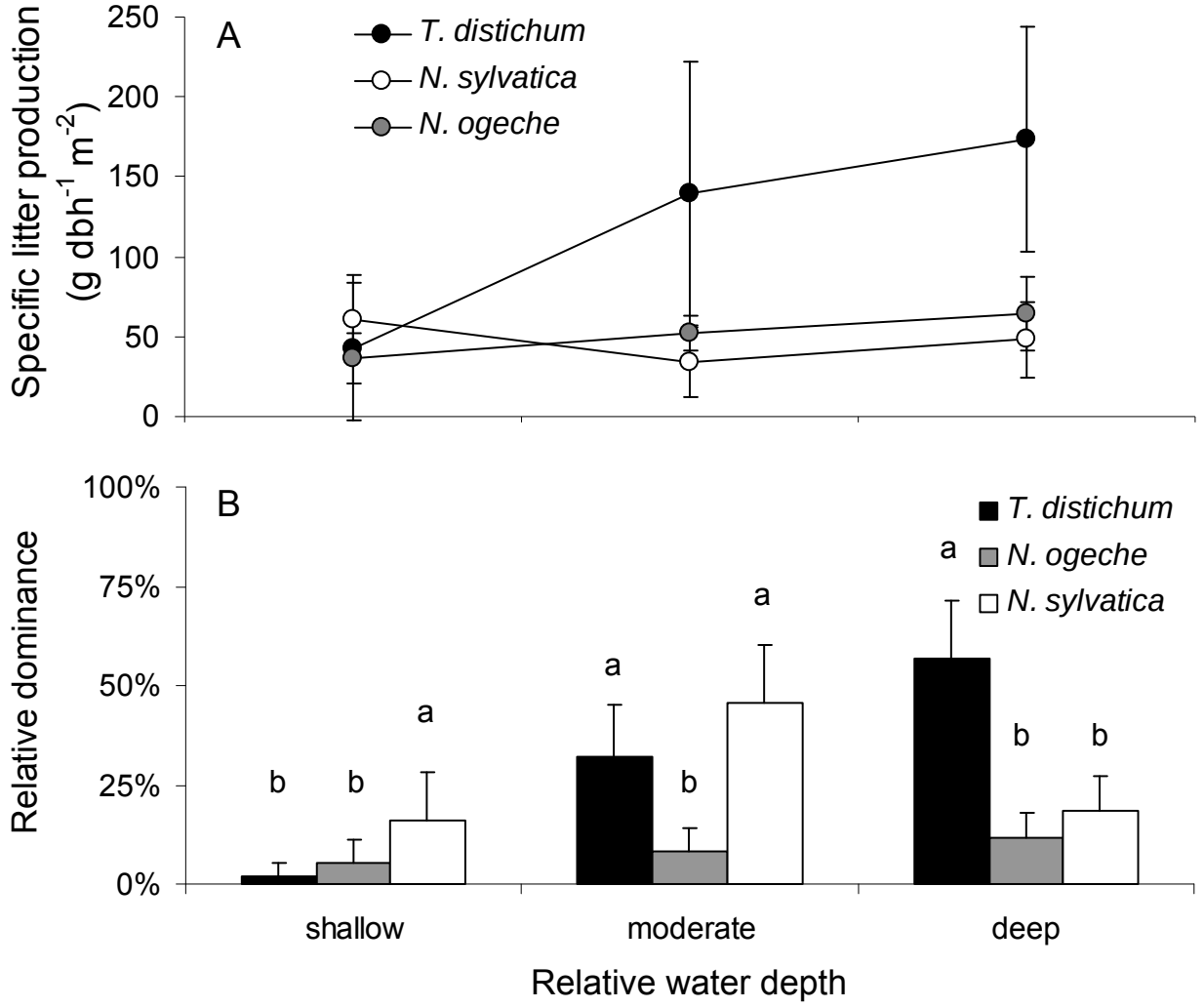
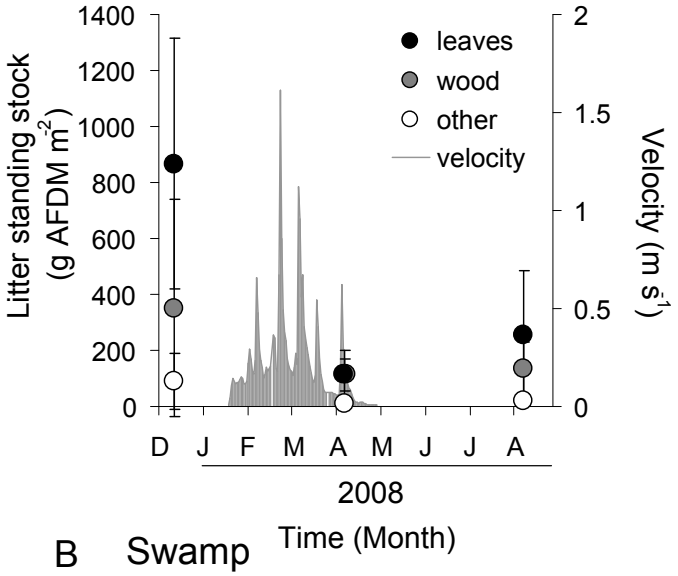

A
T. distichum
N. sylvatica
N. ogeche
Specific litter production
(g dbh-1 m-2)
250
200
150
100
50
0
B
T. distichum
N. ogeche
N. sylvatica
Relative dominance
100%
75%
50%
25%
0%
a
b
b
a
b
a
a
b
b
shallow
moderate
deep
Relative water depth

Figure 4.6: Litter standing stocks (first ordinate) and water velocity (second ordinate) over time in 2007 and 2008 in the A) stream site and B) swamp site in the Little River. Note difference in scale for second ordinate between panels A and B.

A Stream



B Swamp

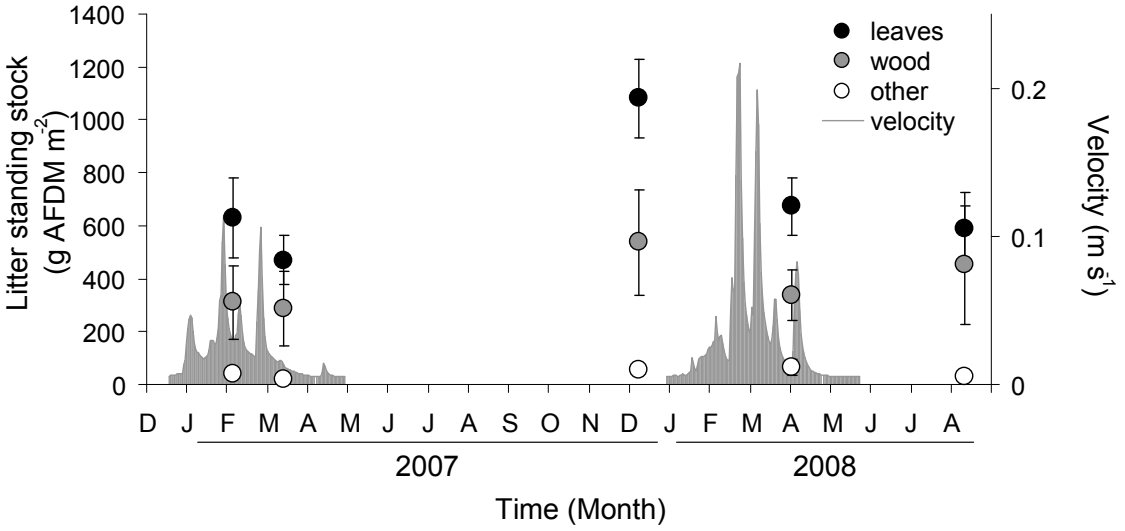


Figure 4.7: Composite oxygen uptake per litter species vs. direct measurements of sediment oxygen demand in A) the stream site and B) the swamp site. Axes in A and B are in different scales.

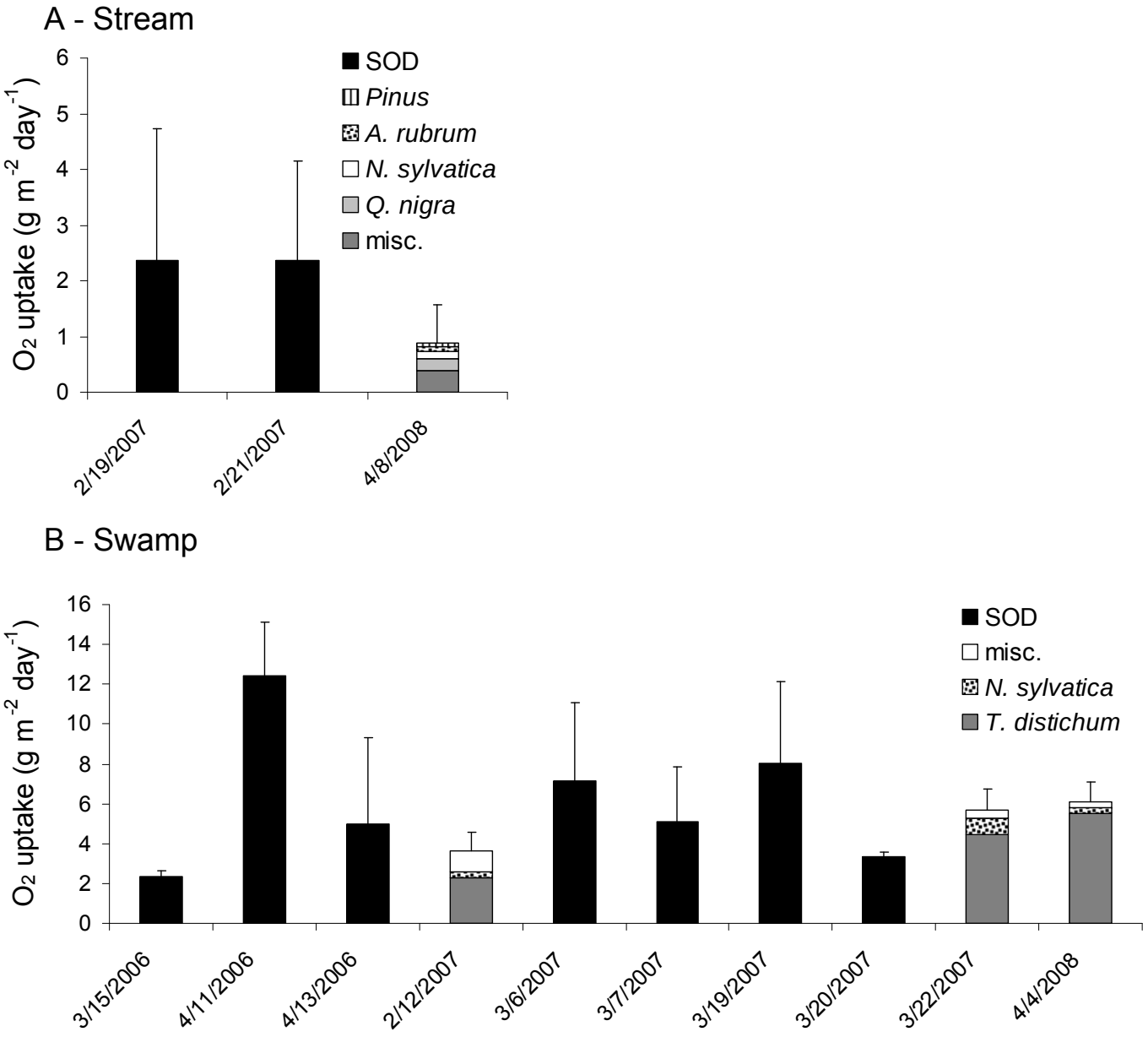
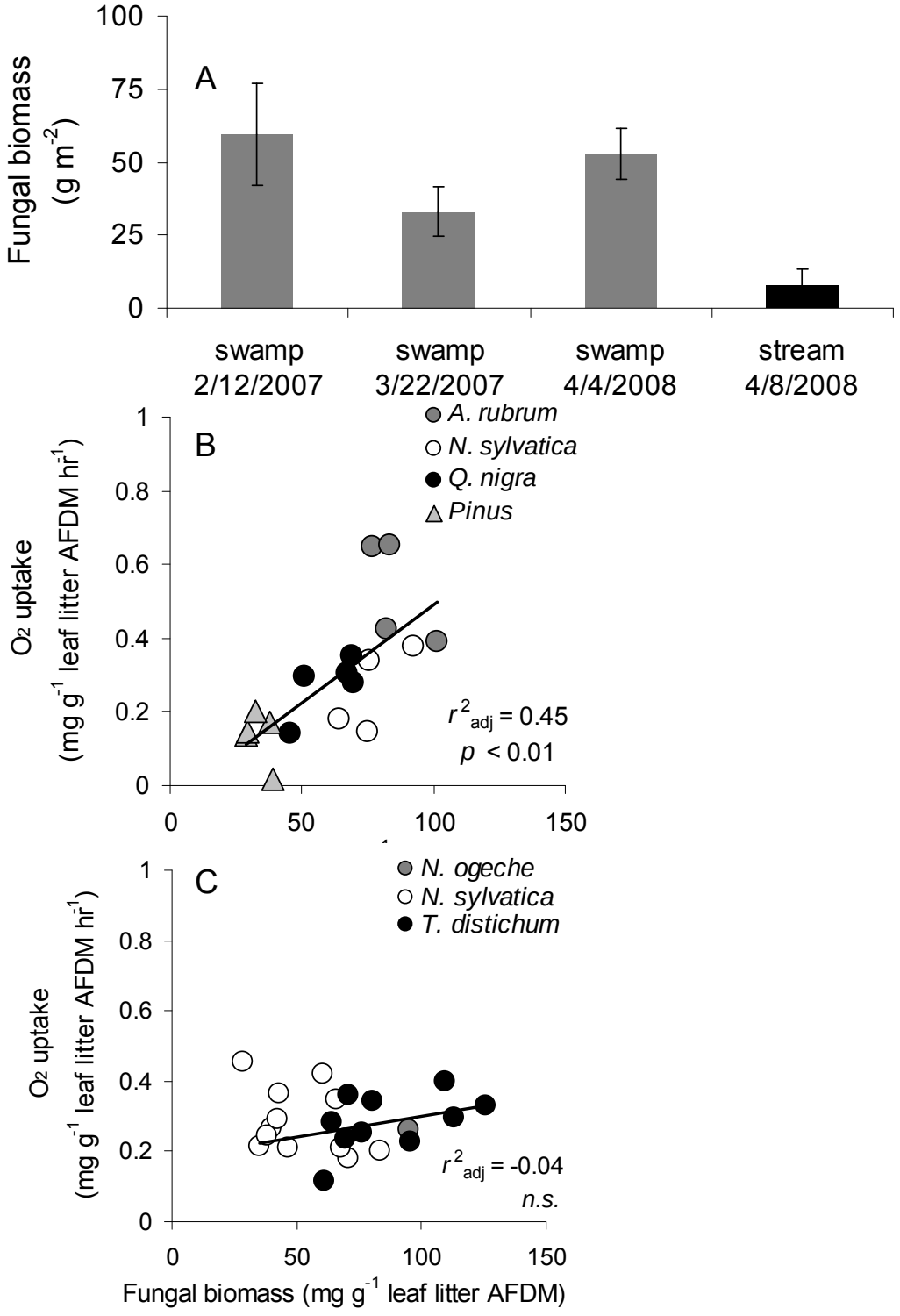

A - Stream
O2 uptake (g m-2 day-1)
0
1
2
3
4
5
6
SOD
Pinus
A. rubrum
N. sylvatica
Q. nigra
misc.
2/19/2007
2/21/2007
4/8/2008
B - Swamp
O2 uptake (g m-2 day-1)
0
2
4
6
8
10
12
14
16
SOD
misc.
N. sylvatica
T. distichum
3/15/2006
4/11/2006
4/13/2006
2/12/2007
3/6/2007
3/7/2007
3/19/2007
3/20/2007
3/22/2007
4/4/2008

Figure 4.8: A) Fungal biomass per m^2 of swamp (gray columns) and stream basin (black column), and B) Fungal biomass vs. temperature-corrected (15˚C) oxygen uptake per leaf litter species in the stream and B) in the swamp.


A
Fungal biomass (g m-2)
100
75
50
25
0
swamp 2/12/2007
swamp 3/22/2007
swamp 4/4/2008
stream 4/8/2008
B
A. rubrum
N. sylvatica
Q. nigra
Pinus
O2 uptake (mg g-1 leaf litter AFDM hr-1)
r2adj = 0.45
p < 0.01
C
N. ogeche
N. sylvatica
T. distichum
r2adj = -0.04
n.s.
Fungal biomass (mg g-1 leaf litter AFDM)

CHAPTER 5

CONSEQUENCES OF DROUGHT ON RIVERINE CARBON CYCLING: IMPLICATIONS OF CLIMATE CHANGE FOR SOUTHEASTERN BLACKWATER RIVERS

[1]A.S. Mehring, R.R. Lowrance, A.M. Helton, G. Vellidis, C.M. Pringle, A. Thompson and D.D. Bosch. To be submitted to *Global Change Biology*.

Abstract

The mechanisms driving large-scale fluctuations in dissolved organic carbon (DOC) concentrations in aquatic ecosystems are subject to debate, with potential drivers including changes in temperature and river discharge as a result of climate change. Projected climate change scenarios for the southeastern United States include decreased rainfall and increased evapotranspiration (ET), both of which are expected to reduce river discharge. Here, we assess the potential impacts of drought on dissolved carbon cycling in a fifth order blackwater river. We use a gradient in rainfall and discharge from a seven-year dataset in the Little River in southern Georgia, USA. DOC bioavailability was investigated across a range of river conditions (i.e. temperature, discharge, dissolved oxygen concentration) through the use of 7-day incubations to measure DOC uptake and CO_2 release. Changes in DOC composition were measured with fluorescence spectroscopy and modeled with parallel factor (PARAFAC) analysis of excitation emission matrices (EEMs). DOC concentrations were negatively correlated to discharge ($F_{1,34} = 9.32$, $p < 0.01$) and positively correlated to temperature ($F_{1,79} = 19.77$, $p < 0.0001$) during fall and winter, and positively correlated to estimated ET during spring ($F_{1,54} = 7.25$, $p < 0.01$). PARAFAC analysis results suggest that DOC in the Little River is primarily composed of three terrestrial humic-like fluorescence groups that do not vary across changes in temperature, discharge or oxygen concentration. Bioavailability and mineralization of DOC were positively correlated to DOC concentrations ($t_{1,43} = 7.19$, $p < 0.0001$, $r^2_{adj} = 0.54$) and field measurements of dissolved oxygen ($t_{1,42} = -3.20$, $r^2 = .20$, $p < 0.01$). Although DOC mineralization rate was negatively correlated to dissolved oxygen concentration, and DOC concentrations were highest (up to 85 mg L^{-1}) during periods of hypoxia, the contribution of DOC mineralization to oxygen demand was relatively small compared to other sources of oxygen demand in the Little River.

Shorter hydroperiods significantly reduced annual downstream export of DOC by approximately eight metric tons for every one-day increase in dry period length ($t_{1,6}$ = -8.71, r^2_{adj} = 0.91, p < 0.001). This may enhance DOC concentrations of future hydroperiods by creating larger stocks of leachable carbon, possibly shifting dissolved carbon cycling toward local mineralization in the face of continuing climate change.

Introduction

Mean concentrations of dissolved organic carbon (DOC) are rising in many aquatic ecosystems around the world and this phenomenon has been explained by an array of causative factors including: decreased ionic strength (Monteith et al. 2007, Hruska et al. 2009), physical disturbance of the terrestrial landscape (Rixen et al. 2008), continuing nitrogen deposition (Findlay 2005), rising CO_2 and temperature (Freeman et al. 2001, Freeman et al. 2004), and decreased river discharge (Eimers et al. 2008a). If, as Larsen et al. (2011) predicts, DOC concentrations continue to increase, broader ecosystem processes and function may be altered (Mulholland 2003). For example, DOC strongly influences microbial production, respiration, and ecosystem metabolism (Findlay et al. 2003, Hanson et al. 2003, Wiegner et al. 2005), and almost half of the carbon inputs to rivers are released to the atmosphere as CO_2 and other greenhouse gases before reaching estuaries (Cole et al. 2007). In aquatic ecosystems already rich in DOC, such as blackwater rivers and wetland-influenced rivers, DO (DO) may reach extremely low levels during periods of low flow and high temperatures (Meyer 1992). Continued increases in temperature projected for the southeastern United States could further reduce riverine oxygen concentrations (Carpenter et al. 1992, Kaushal et al. 2010), while continued increases of DOC

concentrations may trigger threshold shifts in dissolved oxygen (DO) status leading to prolonged states of hypoxia or anoxia (Hamilton et al. 1997, Rixen et al. 2008).

The southeastern United States has experienced significant long-term decreases in warm season precipitation during the past 1000 years (Stahle and Cleaveland 1992, Alexandrov and Hoogenboom 2001). Most high resolution climate change models predict further reductions in rainfall throughout the region, with increasing drought severity (Dai 2010) and potential decreases in summer precipitation of 20-30% (Mearns et al. 2003). Climate models that predict moderate increases in rainfall, still predict increased evapotranspiration (ET) to exceed precipitation and thus reductions in river discharge throughout mid- and low latitudes (Mulholland et al. 1997, Nijssen et al. 2001). In organic-matter-rich, wetland-influenced streams and rivers, DOC concentrations are often high when discharge is low (Mulholland and Kuenzler 1979, Eimers et al. 2008c). This provides a critical link between climate change and carbon cycling, as reduced rainfall and discharge may lead to higher DOC concentrations in some rivers (Eimers et al. 2008a), with potential effects on local ecological processes as well as export to downstream reaches.

Blackwater rivers are globally important suppliers of DOC to the world's oceans (Beck et al. 1974, Raymond and Bauer 2001, Baum et al. 2007), and typically export larger quantities of carbon than other rivers of similar size and discharge (Mulholland and Kuenzler 1979, Schlesinger and Melack 1981). In some rivers, DOC concentration is positively correlated to discharge, and in those cases it is reasonable to expect that DOC export would decrease as river discharge decreases. However, the effects of decreased river discharge on carbon export may be difficult to predict in rivers where DOC concentration is negatively correlated to discharge. For example, decreases in downstream export due to reduced discharge may be partially balanced by

increases in concentration (Eimers et al. 2008b), or may be enhanced through increased mineralization rates (and export as CO_2 or CH_4) during periods of high DOC concentration and high temperatures (Pulliam 1993, Kankaala and Bergström 2004). Much of this depends on the chemical structure and relative rate of consumption by bacteria (bioavailability) of DOC. Determining how DOC export is affected by low flow events is critical to predicting changes in riverine carbon cycling in the face of predicted decreases in river flux in the southeast.

In this study, we seek to evaluate the impact of reduced rainfall and discharge on DOC cycling in a southeastern blackwater river. Our objectives are to: 1) determine the drivers and temporal patterns of DOC composition, concentration, bioavailability, and export, and 2) determine potential relationships between carbon and oxygen cycling, under variable drying regimes in the Little River in south-central Georgia, USA. We accessed a seven-year dataset from a Georgia watershed that encompassed a large gradient in rainfall and discharge. The long-term dataset was also complimented with contemporary (2008-2010) optical analysis of dissolved organic matter (DOM), as well as incubations to characterize changes in composition, bioavailability, and potential mineralization rates.

Methods

Site description – This study was conducted in a heavily forested fifth-order swamp in the Little River, a blackwater river in Tift County, Georgia, USA, draining the Atlantic coastal plain of North America. The study reach is contained within the Little River Experimental Watershed (LREW) (Bosch et al. 2007a). Located in the headwaters of the upper Suwannee River basin, the 33,400-ha LREW was equipped in the 1960's and 1970's for continuous rainfall and stream flow measurements by the Southeast Watershed Research Laboratory (SEWRL) of the United States

Department of Agriculture Agricultural Research Service (USDA-ARS) (Bosch and Sheridan 2007). Currently, the LREW includes eight gauged watersheds ranging in size from 260 to 33,400 ha (Fig. 5.1). Detailed records of rainfall, stream flow, nutrient concentrations, and DO concentrations are regularly collected in each sub-watershed (hourly, daily or weekly depending on sub-watershed and type of data collected).

The study reach (31º 28' 54", 83º 35' 03") drains the entire 33,400-ha catchment, which meanders through a heavily-forested swamp just upstream from the weir. Discharge is highly variable. Like most other headwaters originating within the Tifton Upland of Georgia's coastal plain, the Little River dries completely during the summer and fall months of most years (Meyer 1992). An impermeable layer of clay and rock separates the surface water of the river from the underlying aquifer, uncoupling river discharge from the underlying Floridan aquifer system (Sheridan 1997), but river discharge is highly dependent upon rainwater recharge of the shallow surficial aquifer (Bosch et al. 2003). Summary physical and chemical data are provided in Table 5.1.

Field methods – Data collected at station B, the outlet of the LREW, were used for the analysis of potential drivers of changes in DOC concentration (Fig. 5.1). Stream flow at the site was recorded at 15-minute intervals by a strain gauge pressure-transducer and a digital data logger at a horizontal weir with a V notch center section (Bosch and Sheridan 2007). An additional horizontal weir serves as an auxiliary overflow structure. Weighted average rainfall in sub-watershed B was measured with a network of Fischer-Porter weighing-type digital gauge recorders from 1972-1992, which were replaced in 1992 with tipping-bucket rain gauges (Texas Electronics TE525) that record each 0.254 mm of rainfall at 5 minute increments, as described by Bosch et al. (2007b). DO and temperature were also recorded at 15-minute intervals from

October 2002 (the beginning of a hydroperiod) to June 2009 at station B of the LREW, using submersible DO (Sensorex DO6000) and temperature sensors (Campbell Scientific, CS107). DO and temperature were also measured weekly at the weir using a hand-held YSI 6600 V2 Sonde with a rapid pulse technology (RPT) DO probe. Automated and hand-held measurements of DO and temperature were averaged when both were available.

Flow-proportional composite water samples were collected from January 2003 through 2010 using an automated ISCO model 3710 sampler at Station B, which collected and deposited water samples in a refrigerated housing (maintained at 4º C) as described by Feyereisen et al. (2007). These samples were returned to the laboratory for analysis of….. Additional grab samples were collected at the weir for analysis of chlorophyll a (chl_a). Estimated ET rates (estimated using the Priestley-Taylor equation) were obtained from the Georgia Automated Environmental Monitoring Network's weather station in Tifton, Georgia, located approximately 6 km east of the watershed outlet.

River depth and water volume per m^2 were estimated by measuring water depths in the swamp throughout the wet season at 50 points evenly spaced (25 m apart) along five transects roughly 100 m apart, running perpendicularly across the study reach channel. By measuring depth throughout the wet season at a range of discharges, average water volume within the swamp (volume per m^2) was calculated from discharge recorded at the weir.

Chemical Analysis – In the laboratory, river water samples were filtered through pre-ashed 0.45-µm glass fiber filters (Whatman, 934-AH) and immediately frozen and stored at -20°C until analysis. DOC was analyzed with a Shimadzu 5050A Total Organic Carbon Analyzer, using the high-temperature combustion method (method 5310B, APHA 1999). Dissolved NO_3^- + NO_2^-–N, NH_4^+–N, Cl^-, and PO_4^{3-}-P using EPA-approved colorimetric techniques (APHA 1999).

Total kjeldahl N and total P were determined on digests of unfiltered samples using Lachat flow injection analyzers (Lachat Instruments, 1997). Chl_a was determined by a method based on USEPA Method 445.0 (USEPA 1998) and Standard Method 10200H (APHA 1999) using a Turner Designs TD700 Fluorometer with optical kit, chlorophyll a standard set, and a solid reference standard (TurnerDesigns 1999).

DOC bioavailability assays – Seven-day, dark, oxic laboratory DOC bioavailability assays were conducted using methods described in McDowell et al. (2006), in which initial and final DOC concentrations as well as CO_2 release were measured. Each sample of river water (20 mL) was filtered into an acid washed and autoclaved 160-mL serum bottle (hereafter referred to as "chamber"), through a sterile 0.2-µm polyethersulfone membrane (Whatman Puradisc™) mounted on a glass syringe. Five mL of filtered sample was immediately acidified and analyzed for DOC using methods described above. Mean sample DOC concentration was 0.66 ppm lower after filtration through 0.2 µm filters (compared to 1 µm filters), but this difference was not significantly different (95% confidence interval = 1.12, n = 45). 150 µL of microbial inoculum (unfiltered river water collected on June 8, 2009) was added to each chamber, as well as 30 µL of nutrient solution (0.1% NH_4NO_3 + 0.1% K_2HPO_4) to prevent limitation by N, P, or K. Blanks were run as deionized water with added nutrients and inoculum as described above. Chambers were immediately sealed with gas-tight septa caps, and were kept in a dark incubation chamber maintained at 20ºC for seven days and agitated once every 24 hours.

At the end of the seven day period, a gas-tight syringe was used to sample 1 cc of gas from within the headspace of each chamber. Samples were analyzed for CO_2 on a Shimadzu GC-2014 Greenhouse Gases Analyzer with a methanizer/flame ionization detector. After gas samples were removed from chambers, caps were removed and 5 mL of water from within the chamber

was again filtered through a sterile 0.2-μm polyethersulfone membrane (Whatman Puradisc™), acidified with HCl and analyzed for DOC. DOC uptake rate (ΔDOC) was calculated using equation 1:

$$\Delta DOC = \frac{DOC_{initial}\,(ppm) \; - \; DOC_{final}\,(ppm)}{incubation\ time\ \ (days)} \qquad (1)$$

DOC mineralization rate (ΔCO_2-C) was calculated as the difference between treatment and blank chamber headspace CO2 concentrations, using equation 2:

$$\Delta CO_2{-}C = \frac{CO_2{-}C_{treatment}\,(ppm) \; - \; CO_2{-}C_{blank}\,(ppm)}{time\ (days)}) \qquad (2)$$

DOC bioavailability was expressed both as percent consumed (ΔDOC divided by initial DOC) and as percent respired (ΔCO_2-C divided by initial DOC).

DOC optical analysis – Fluorescence spectroscopy was used to measure Emission-Excitation Matrices (EEMs) for DOC samples collected between January 2008 and May 2010 (Fluorlog-3; Horiba, Jobin Yvon; University of North Carolina-Chapel Hill) (5 nm slit width), by measuring fluorescence intensity across excitation wavelengths 240 to 450 nm (5 nm increment) and across emission wavelengths 140 to 950 nm (1 nm increment) for each sample. Fluorescence index (FI), which is used as an indicator of terrestrial or aquatic microbial origin of DOC, was calculated as the ratio of emission intensity at 450 nm to that at 500 nm, obtained at an excitation wavelength of 370 nm (McKnight et al. 2001). All samples were diluted 1:3 with laboratory-grade DI water, and fluorometer integration times were adjusted between 0.7-3.5 seconds depending on DOC concentration. We corrected EEMs for inner-filter effects and for instrument-specific excitation and emission corrections in MATLAB R2008b (Mathworks) following Cory et al. (2010) via a user-generated rhodamine spectrum for excitation correction (DeRose et al. 2007) and a manufacturer provided emission correction spectrum specific to the CCD detector

(Horiba Scientific). We subtracted similarly analyzed blank EEMs, collected from laboratory-grade DI water, from sample EEMs. Fluorescence intensities in blank-corrected sample EEMs were converted to Raman units (Stedmon et al. 2003).

Parallel Factor Analysis (PARAFAC), a multivariate data analysis technique, was conducted to decompose blank-corrected EEMs into unique fluorescence groups representing chemically independent components that describe the total EEM (Stedmon et al. 2003). For each EEM, components are described by F_{max} values. Because the chemical structure of the components is unknown, F_{max} values represent the maximum fluorescence of each component, rather than the actual concentration. PARAFAC was conducted according to Stedman and Bro (2008) using the DOMFluor toolbox in MATLAB R2008b (Mathworks).

UV-visible absorbance was measured on diluted (1:3, as above) samples at 254 nm using a 1-cm path length quartz cuvette for each sample on a Perkin Elmer 559 UV/Visable Spectrophotometer and specific UV absorbance ($SUVA_{254}$) was calculated by dividing UV absorbance by the cuvette path length (in meters) and normalizing to DOC concentration (mg L^{-1}) (APHA 1999). $SUVA_{254}$ is a measure of aromatic carbon content of the DOC (Weishaar et al. 2003), with smaller $SUVA_{254}$ values (< 2 mg-C^{-1} m^{-1}) associated with low aromaticity. Average absorbance of diluted samples was 0.23 ± 0.02 AU (± 1 99% C.I.), with a maximum absorbance of 0.50 AU.

Statistical analysis – All datasets were analyzed with simple or multiple linear regression (PROC REG at $\alpha = 0.05$) except for changes in DOC bioavailability (percent consumed or respired) over time, which were analyzed with analysis of covariance (ANCOVA, PROC GLM at $\alpha = 0.05$) in SAS version 9.2 (SAS Institute Inc., Cary, USA). Data were log-transformed whenever necessary to meet assumptions of normality, homogeneity of variance, and linearity.

When testing for changes in annual dry period length between 1972-2009, the *t* value was computed using the heteroskedasticity consistent covariance matrix described by White (1980) (proc reg, option=acov), due to an increasing variance over time that was indicated by White's test (proc reg, option=spec, SAS version 9.2 [SAS Institute Inc., Cary, USA]). The same matrix correction was used when testing for the effect of DOC exports from the previous hydroperiod on DOC concentrations of the current hydroperiod.

To isolate the set of factors among temperature, discharge, and DOC concentration best explaining changes in oxygen concentration during hydroperiods, we used Akaike's Information Criterion (AIC) and the information theoretic approach (Burnham and Anderson 2002) to select the most plausible multiple regression models based on lowest AIC_C (AIC corrected for small sample size, selected based on lowest Mallows' Cp) using PROC REG at $\alpha = 0.05$ in SAS version 9.2 (SAS Institute Inc., Cary, USA). A candidate model was considered plausible if the difference between it's AICc score and that of the top model (Δi) was less than 10. Models with $\Delta i > 10$ were rejected. For all multiple regression analyses, explanatory variables were tested for multicollinearity by examining Pearson's correlation coefficient matrices and calculating variance inflation factors (VIF). If two variables exhibited high VIF (> 2.5) and/or cross-correlation ($r > 0.60$), two separate multiple regression models were run, with one of the two cross-correlated variables removed from each model. The average VIF in final models did not exceed the total number of explanatory variables (Neter et al. 2004), nor did it exceed 2.5 in any single parameter.

When comparing the effects of temperature, discharge, and ET on DOC concentration, the time series was split among seasons, which were delineated by solstices and equinoxes. When analyzing the effect of previous DOC exports on current concentrations, the time series

was split into six hydroperiods. One hydroperiod ended when river flow ceased for more than two weeks. Dry period length was calculated as the total number of days during which the river did not flow within a given year. In most years this was an uninterrupted span of time, but in 2008 a dry period lasting from May 25-October 23 was interrupted by a brief hydroperiod from August 23-September 20.

Results

DOC and oxygen – Temperature, DO, DOC concentration, and discharge are correlated in the Little River (Fig. 5.2). DOC concentration was negatively correlated with discharge when analyzed across the time series ($t_{1,232}$=-6.998, r^2_{adj}=0.23, Fig. 5.3), with a relationship similar to those observed in boreal peatland streams (Eimers et al. 2008a). Discharge explained the most variability in DOC concentration during fall and winter months (Fig. 5.2, Table 5.2). DOC concentrations decreased over time during fall and winter, reaching an annual minimum between early December and mid-February, but rising with increasing temperature in late winter (Fig. 5.2, Table 5.2). DOC concentration was positively correlated with ET and continued to rise during spring. During summer, DOC concentration was higher and more variable than in any other season (Fig. 5.2, Table 5.2), but little of the variation could be explained by changes in temperature, discharge, or ET. In all seasons, DOC concentration during the previous week explained a significant proportion of the variability in current DOC concentration.

Dissolved oxygen concentration was lowest at the beginning and end of hydroperiods, and correlated with temperature, discharge, and DOC concentration across the full time series

($F_{3,172} = 260.49$, $p < 0.0001$, $R^2_{adj} = 0.82$) (Fig. 5.2). Regression models omitting any one of the three parameters were rejected based on a higher degree of error associated with simpler models ($\Delta AIC_C > 20$). During the fall months DO concentration increased over time and was negatively correlated to temperature and positively correlated to discharge (Fig. 5.2, Table 5.3). In early winter DO concentration increased over time and reached the highest average concentrations (Fig. 5.2, Table 5.3), but gradually decreased over time in late winter and spring. In both winter and spring DO was negatively correlated to temperature and DOC concentration, and positively correlated to discharge, but DOC was most strongly correlated to DO in winter (Table 5.3). In summer, DO concentration was at its lowest, and although it was positively correlated to discharge, little of the variability was explained by the measured parameters (Table 5.3). Concentrations of DOC and DO were not correlated to chloride, N or P concentrations.

Rainfall, dry period length, and downstream exports – Annual DOC exports and total days without flow (dry period length) had a significant negative linear relationship between 2003-2009 ($t_{1,6} = -8.71$, $r^2_{adj} = 0.91$, $p < 0.001$, Fig. 5.4A), with a decrease in export of approximately eight metric tons of DOC for each one-day increase in annual dry period length. Higher DOC exports in a given hydroperiod were significantly correlated with lower minimum ($t_{1,4} = -7.08$, $r^2_{adj} = 0.89$, $p < 0.01$) and average ($t_{1,4} = -6.01$, $r^2_{adj} = 0.87$, $p < 0.01$) DOC concentrations in the following hydroperiod (Fig. 5.4B). Variability in hydroperiod average and minimum DOC concentrations were not correlated to the average temperature or river discharge of the same hydroperiod.

The study period (2003-2009) encompassed the range of flow conditions observed since 1972 (Fig. 5.5A), with 2007 being the driest year on record since 1972 (no flow for 65% of the year), but also with continuous flow from October 31, 2002 until May 25, 2004. Annual rainfall

decreased significantly from 1972-2009 ($t_{1,36}$ = -2.33, $p < 0.05$, $r^2_{adj.}$ = 0.11). Annual dry period length increased during the same time period ($t_{1,36}$ = 2.01, p = 0.052, $r^2_{adj.}$ = 0.09, Fig. 5.5A) as did the variability of dry period length (White's test, x^2_2 = 6.35, p = 0.042), with differences in annual rainfall explaining a significant proportion of the variability ($t_{1,36}$ = -6.95, $p < 0.0001$, $r^2_{adj.}$ = 0.56). However, maximum dry period length per 5-year increment experienced dramatic increases between 1972 and 2009 ($t_{1,6}$ = 6.34, $p < 0.001$, $r^2_{adj.}$ = 0.85, Fig. 5.5B). Minimum annual rainfall per 5-year increment explained 88% of the variability in maximum dry period length ($t_{1,6}$ = -7.08, $p < 0.0001$, $r^2_{adj.}$ = 0.88, Fig. 5.5B), and decreased significantly during the same time period ($t_{1,6}$ = -14.90, $p < 0.0001$, $r^2_{adj.}$ = 0.97, Fig. 5.5B).

DOC composition, mineralization and bioavailability – Based on fluorescence analysis, the overall chemical composition of DOC in the Little River changed little between 2008-2009, and was stable throughout increases in DOC concentration that *preceded* dry periods. Three fluorescent components were identified with the PARAFAC model after removal of outlier EEMs (Fig. 5.6, Table 5.4), and models were validated using the split-half and random initialization methods (Stedmon and Bro 2008). Comparison of modeled components to previously published PARAFAC models reveals that all three DOC components have characteristics similar to terrestrial humic-like material found in forested streams and wetlands free of wastewater (Table 5.4). Components were positively correlated with each other ($r^2_{adj} \geq$ 0.95, p<0.0001) and with bulk DOC measurements ($r^2_{adj} \geq 0.74$, p<0.0001). Amino acid-like components were not detected, but additional uncharacterized chemical components may be present in DOC immediately *following* dry periods, during the inundation of river channels. Four of the five outlier EEMs were generated from water samples collected immediately after flow returned to the channel. Their unique matrices hindered PARAFAC model validation and

necessitated exclusion from our analysis. However, fluorescence index (FI) of all samples (including PARAFAC outliers) ranged from 1.32 to 1.48, indicative of material of terrestrial detrital (rather than aquatic microbial) origin (McKnight et al. 2001).

DOC mineralization rates were highest at the beginning and end of hydroperiods (Fig. 5.7A), when discharge was low and DOC concentrations were high. DOC mineralization rates were positively correlated with DOC concentration ($t_{1,43} = 7.19$, $p < 0.0001$, $r^2_{adj} = 0.55$, Fig. 5.7B) and consumption rates ($t_{1,43} = 5.36$, $p < 0.0001$, $r^2_{adj} = 0.39$). Similarly, DOC consumption rates were also positively correlated with DOC concentration ($t_{1,43} = 7.52$, $p < 0.0001$, $r^2_{adj} = 0.57$, Fig. 5.7B). However, estimated CO_2 release on an areal basis (per m^2) was less variable and was highest at high flows, averaging 0.22 ± 0.03 (± 1 s.e.) and ranging 0.07-1.05 g CO_2 m^{-2}.

Average DOC bioavailability was similar whether expressed as percent consumed (19.39 ± 0.01 [± 1 s.e.]) or percent respired (14.39 ± 0.01 [± 1 s.e.]), with values ranging from -2 to 37% consumption or respiration of initial carbon concentrations for a given sample throughout the 7 day incubation. There was no significant difference in DOC bioavailability when expressed as percent consumed between years ($F_{1,19} = 0.43$, $p = 0.52$), or among months ($F_{1,19} = 3.01$, $p = 0.099$) in 2008 and 2009. The percentage of DOC respired also did not differ significantly between years ($F_{1,19} = 0.08$, $p = 0.78$), but differences did exist among months ($F_{1,19} = 4.45$, $p < 0.05$, Fig. 5.8A). DOC bioavailability (percent respired) was also negatively correlated with field measurements of DO concentration ($t_{1,42} = -3.20$, $r^2 = .20$, $p < 0.01$). $SUVA_{254}$, an indicator of percent aromaticity (high values indicate a higher relative abundance of aromatic rings), was negatively correlated with both ΔDOC (mg L^{-1} day^{-1}) ($t_{1,43} = -5.07$, $p < 0.0001$, $r^2_{adj} = 0.37$) and ΔCO_2-C (mg L^{-1} day^{-1}) ($t_{1,43} = -4.88$, $p < 0.0001$, $r^2_{adj} = 0.35$, Fig. 5.8B). The dry-wet transition

period samples had among the lowest $SUVA_{254}$ values of all samples (Fig. 5.8B), consistent with fluorescence analysis that suggested unique chemical properties of these samples.

Discussion

Climatic conditions influence DOC export and mineralization – Our results illustrate strong effects of local climatic conditions on carbon cycling in southeastern blackwater rivers, the most compelling being the effects of drought and river drying on carbon exports and mineralization. With decreased annual rainfall and river discharge, riverine DOC export to downstream reaches was significantly curtailed. This was positively correlated to increased average and minimum DOC concentrations in the following hydroperiod, similar to what is observed following droughts in Mediterranean streams (Vázquez et al. 2007). Because DOC concentration was positively correlated to mineralization rate in our laboratory incubations, reduced DOC export brought about by long dry periods may result in increases in local DOC mineralization rates during wet periods. In the Little River, large stocks of leaf litter remain in river channels after short hydroperiods (Mehring 2012). This accumulated organic matter, from which more DOC can be generated, may be a mechanism behind increased DOC concentration following a short hydroperiod.

Laboratory assays suggest that DOC during wet-dry transition phases is more bioavailable. Although fluorescence index values suggested that all DOM samples were dominated by DOC of terrestrial non-microbial origin, DOM during the transition period between…. was rapidly respired in our bioavailability studies (Fig. 5.8B) and exhibited the lowest SUVA and unique fluorescence emission values of all samples examined. This is

consistent with a highly-labile DOM with fewer aromatic moieties and likely a lower molecular weight than the DOM present during the rest of the study. Collectively, these results suggest that decreasing downstream export and increasing local mineralization may occur during drier years. Meyer (1992) noted that the majority of upper reaches in Georgia coastal plain blackwater rivers dried completely during many summer months, and therefore the carbon dynamics observed in the Little River may not be unique among blackwater systems.

DOC and hypoxia in blackwater rivers – DOC is often the suspected cause of hypoxia in wetland-draining?? and blackwater rivers (Hamilton et al. 1997, Rixen et al. 2008), and also during blackwater events, in which abnormal pulses of high concentration DOC water is introduced to a system (Hladyz et al. 2011). In the Little River, times of high DOC concentration often coincided with periods of hypoxia, and potential bioavailability and mineralization rates of DOC samples were negatively correlated with field measurements of DO. In laboratory incubations, DOC concentration, the aromatic character of the DOC (e.g., $SUVA_{254}$ values), and potential mineralization rates were strongly correlated. However, DOC may be a less significant contribution to hypoxia, relative to other oxygen consuming processes in the Little River. In the same study reach, leaf litter alone was estimated to consume 5.54 g O_2 m^{-2} day^{-1} (Mehring 2012), and Todd et al. (2009) estimated total benthic oxygen demand to be 6.20 g O_2 m^{-2} day^{-1}. Assuming a respiratory quotient of 1, areal oxygen demand generated by DOC turnover in the water column averaged only 3.99 ± 0.92% (± 1 95% C.I.) that of average oxygen demand generated by leaf litter-associated microorganisms.

This should not be interpreted as a lack of connection between DOC and oxygen dynamics within the Little River. Although water column respiration was low compared to that generated by benthic detritus, the respiration of microorganisms on leaf litter and within

hyporheic zones may be highly dependent on DOC as source of carbon (Findlay et al. 2003, Wiegner et al. 2005). There is also a possibility that DOC spikes may be the consequence, rather than the cause, of extreme fluctuations in oxygen availability in the Little River. During summer months, when temperatures were highest and the river was often hypoxic or anoxic, DOC concentration was highest and most variable. DOC fluctuations in summer could be partially driven by iron (Fe) and manganese (Mn) reduction likely to occur under those conditions, potentially allowing for release of bound DOC from Fe and Mn co-precipitates (Skoog and Arias-Esquivel 2009, Burgin et al. 2011).

Additional geologic, hydrologic, and human influences - Rainfall in the LREW has declined since 1972, but anthropogenic drivers may also influence the hydrology of the system. Although the Little River is separated from deep aquifers by relatively impermeable geological formations, the hyporheic zone provides considerable water storage capacity and creates a surficial aquifer (Shirmohammadi et al. 1986). When this surficial aquifer is filled to capacity, baseflow conditions may last into the summer with occasional recharge by summer storms (Shirmohammadi et al. 1986, Bosch et al. 2003). However, the negative effects of decreased precipitation on river discharge may be exacerbated by recent increases in farm ponds (De Steven and Lowrance 2011) and irrigation (Mullen et al. 2009) throughout the region. While farm ponds are not likely to have an impact during periods of adequate rainfall, impoundment of water and withdrawal for irrigation during the growing season likely contribute to low flows (Lowrance et al. 2007), thereby lengthening dry periods. Following droughts, ponds withhold water that would otherwise enter the surficial aquifer, which must be recharged before significant river flow can resume. Because farm ponds are being constructed for irrigation during warmer months of the year when precipitation is already low, river flow may be additionally impacted.

Seasonal influence on DOC concentration and the effects of climate change – The seasonally-variable drivers of DOC (temperature, discharge, and ET) in the Little River are all susceptible to the shifts in environmental conditions, such as increased temperature and decreased precipitation, which are predicted to accompany climate change. Two critical events coincide to drive DOC concentrations upwards during the Fall and Winter seasons: 1) the inundation of channels after long dry periods, and 2) fresh inputs of leaf litter just prior to or during inundation, providing a source of leachable carbon (Hamilton et al. 1997, Meyer et al. 1998, Vázquez et al. 2007). As winter progresses in the Little River, rising temperatures may allow for enhanced microbial activity and additional release of DOC. In Spring, ET rates increase with rising temperature and water uptake by riparian and riverine trees (Szilagyi 2000), potentially increasing DOC concentration. If winter temperatures continue to rise with further warming, so may the enhancement of microbial DOC release by rising temperatures. Also, as both primary production and ET rates may increase with further increases in atmospheric CO_2 and temperature (Melillo et al. 1993, Mulholland et al. 1997, Nemani et al. 2003), ET's concentrating effects on water column solutes may be enhanced. As decreased export during short hydroperiods appeared to increase DOC concentrations in the following hydroperiod, further predicted decreases in rainfall (Mearns et al. 2003), intensifying droughts (Dai 2010), and reduced river discharge (Mulholland et al. 1997) may raise DOC concentrations during times when water is present, as large stocks of leaf litter and leachable carbon are generated (Vázquez et al. 2007). Rivers serve as sites of considerable organic matter processing (Cole et al. 2007) and also as conduits transporting DOC from headwaters to oceans. Through continued changes in the atmospheric conditions influencing both processes, the potential exists for perturbation of

ecosystem function across broad longitudinal scales, ultimately affecting CO_2 emission from aquatic ecosystems and DOC delivery from freshwater to marine ecosystems.

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Table 5.1: Average physical and chemical data (mean ± 1 S.E.) within the Little River Experimental Watershed (LREW) between 2003 and 2009. Minimum (min) and maximum (max) values are provided for each parameter, as well as sample size (n). Minimum (below detection = *bd*) and maximum values for DO and temperature are from daily averages of data collected at 15-minute intervals.

Parameter				min	max	n
DO	2.67	±	0.05 mg L^{-1}	0.19	9.26	1560
DOC	21.86	±	0.74 mg L^{-1}	8.04	85.96	235
pH	7.19	±	0.03	6.18	8.18	203
temperature	16.94	±	0.14 °C	3.11	29.54	1884
chlorophyll a	7.61	±	0.31 µg L^{-1}	*bd*	17.30	131
P (total)	67.43	±	8.29 µg L^{-1}	*bd*	637.00	202
PO_4^{3-}	10.34	±	1.15 µg L^{-1}	*bd*	118.00	230
N (TKN)	441.40	±	33.29 µg L^{-1}	*bd*	2600.00	201
NO_3^-	59.00	±	6.86 µg L^{-1}	*bd*	602.60	232
NH_4^+	31.77	±	2.86 µg L^{-1}	*bd*	263.50	234
Cl	11.66	±	0.26 mg L^{-1}	1.11	23.10	234

Table 5.2: Stepwise multiple regression of DOC concentration against discharge, temperature, evapotranspiration (ET), and previous DOC concentration (DOC lag) separated by season. For each season, average DOC concentration (± 1 95% C.I.) and the full model's coefficient of determination (Model R^2_{adj}, adjusted for sample size) are provided. Statistical test results are also provided for individual parameters.

Season	**DOC mg L^{-1}**	**Model R^2_{adj}**	**Discharge**	**Temperature**	**ET**	**DOC lag**
Fall (Sep. 22-Dec. 20)	23.41 (0.49)	0.47	$F_{1,34} = 9.32$ $p < 0.01$ **(-)**	*ns*	*ns*	$F_{1,34} = 9.00$ $p < 0.01$
Winter (Dec. 21-Mar. 19)	16.97 (1.52)	0.76	$F_{1,79} = 5.10$ $p < 0.05$ **(-)**	$F_{1,79} = 19.77$ $p < 0.0001$ **(+)**	*ns*	$F_{1,79} = 180.30$ $p < 0.0001$
Spring (Mar. 20-June 20)	23.59 (3.06)	0.65	*ns*	*ns*	$F_{1,54} = 7.25$ $p < 0.01$ **(+)**	$F_{1,54} = 85.86$ $p < 0.0001$
Summer (June 21-Sep. 21)	34.97 (11.96)	0.26	*ns*	*ns*	*ns*	$F_{1,37} = 14.60$ $p < 0.001$

Table 5.3: Stepwise multiple regression of DO concentration against DOC, discharge, and temperature separated by season. For each season, average DO concentration (± 1 95% C.I.) and the full model's coefficient of determination (Model R^2_{adj}, adjusted for sample size) are provided. Statistical test results are also provided for individual parameters.

Season	**DO mg L^{-1}**	**Model R^2_{adj}**	**DOC**	**Discharge**	**Temperature**
Fall (Sep. 22-Dec. 20)	2.73 (0.76)	0.55	*ns*	$F_{1,34} = 4.70$ $p < 0.05$ **(+)**	$F_{1,34} = 40.69$ $p < 0.0001$ **(-)**
Winter (Dec. 21-Mar. 19)	4.63 (0.36)	0.73	$F_{1,76} = 77.37$ $p < 0.0001$ **(-)**	$F_{1,76} = 47.58$ $p < 0.0001$ **(+)**	$F_{1,76} = 26.73$ $p < 0.0001$ **(-)**
Spring (Mar. 20-June 20)	1.90 (0.39)	0.71	$F_{1,53} = 5.61$ $p < 0.05$ **(-)**	$F_{1,53} = 54.92$ $p < 0.0001$ **(+)**	$F_{1,53} = 20.88$ $p < 0.0001$ **(-)**
Summer (June 21-Sep. 21)	1.44 (0.39)	0.16	*ns*	$F_{1,38} = 8.48$ $p < 0.01$ **(+)**	*ns*

Table 5.4: Characterization of three PARAFAC components of DOM identified in this study, compared with previously identified components. All three components resemble terrestrial humic-like material. Maximum excitation ("Ex. Max.", secondary excitation peaks in parentheses) and emission wavelengths ("Em. Max.") are provided. Component descriptions are based on similar excitation and emission maxima of components in the provided references.

Component	Ex. Max. (nm)	Em. Max. (nm)	Description	References
1	<240 (280)	418	Forested streams and wetlands, absent in wastewater	P3(<260,380/498) - (Murphy et al. 2008) C3(<250,305/412) – (Stedmon and Markager 2005); C2 (<250,305/412) – (Stedmon et al. 2003); A (260/380-460) – (Coble 1996)
2	<240 (350)	468	Natural and agricultural catchments, absent in wastewater	C1(<250/448) - (Stedmon and Markager 2005) C1(<250/448) – (Stedmon et al. 2003);
3	<240 (270) (410)	495	Widespread, high molecular weight fraction	C3 (260,370/490), P3 (<260,380/498) - (Murphy et al. 2008) C3 (270,360/478) – (Stedmon et al. 2003);

Figure 5.1: Map of the Little River Experimental Watershed (LREW). Letters indicate locations of monitoring stations in each shaded sub-watershed. Water samples for the current study were collected at sub-watershed B, which is the outlet for the entire LREW.

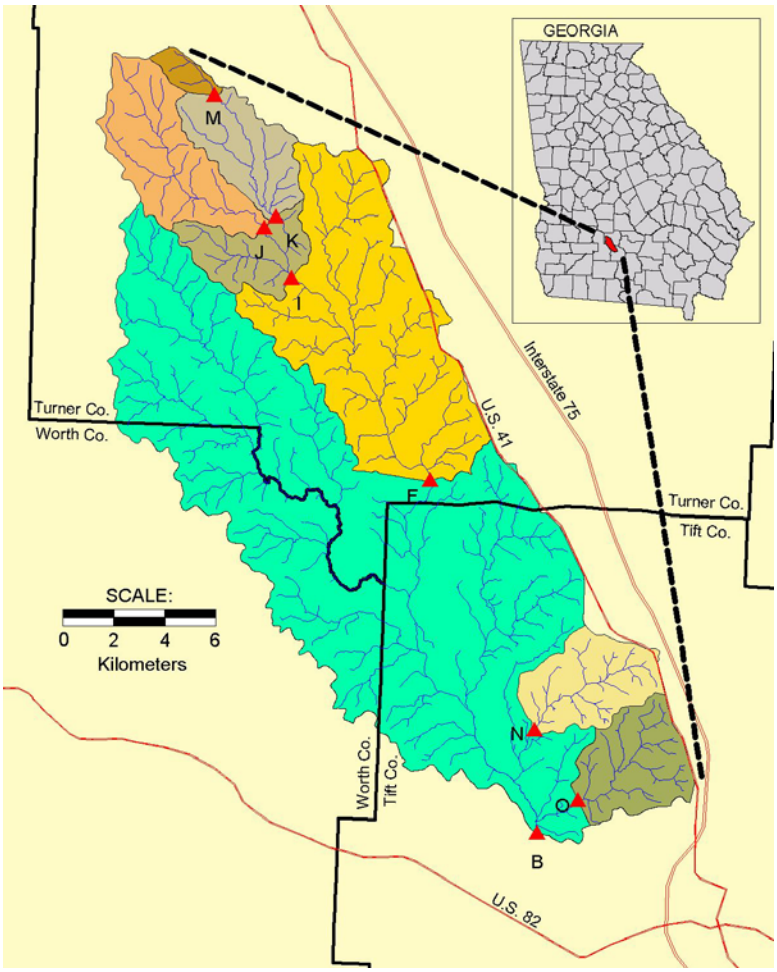

GEORGIA
M
K
J
I
Interstate 75
U.S. 41
Turner Co.
Worth Co.
F
Turner Co.
Tift Co.
SCALE:
0 2 4 6
Kilometers
N
O
B
Worth Co.
Tift Co.
U.S. 82

Figure 5.2: A) Dissolved organic carbon (DOC) concentration (mg L^{-1}, white circles) and export (kg, gray circles), B) dissolved oxygen (DO, mg L^{-1}), temperature (ºC), and C) discharge (L s^{-1}, logarithmic scale) over time from 2003-2009. Average daily values are provided for temperature, dissolved oxygen, and discharge, while DOC is a flow-weighted composite sample (approximately average) from a ~7-day sampling period. Export was calculated by multiplying composite DOC sample concentration times the total liters of water discharged during the sampling period.

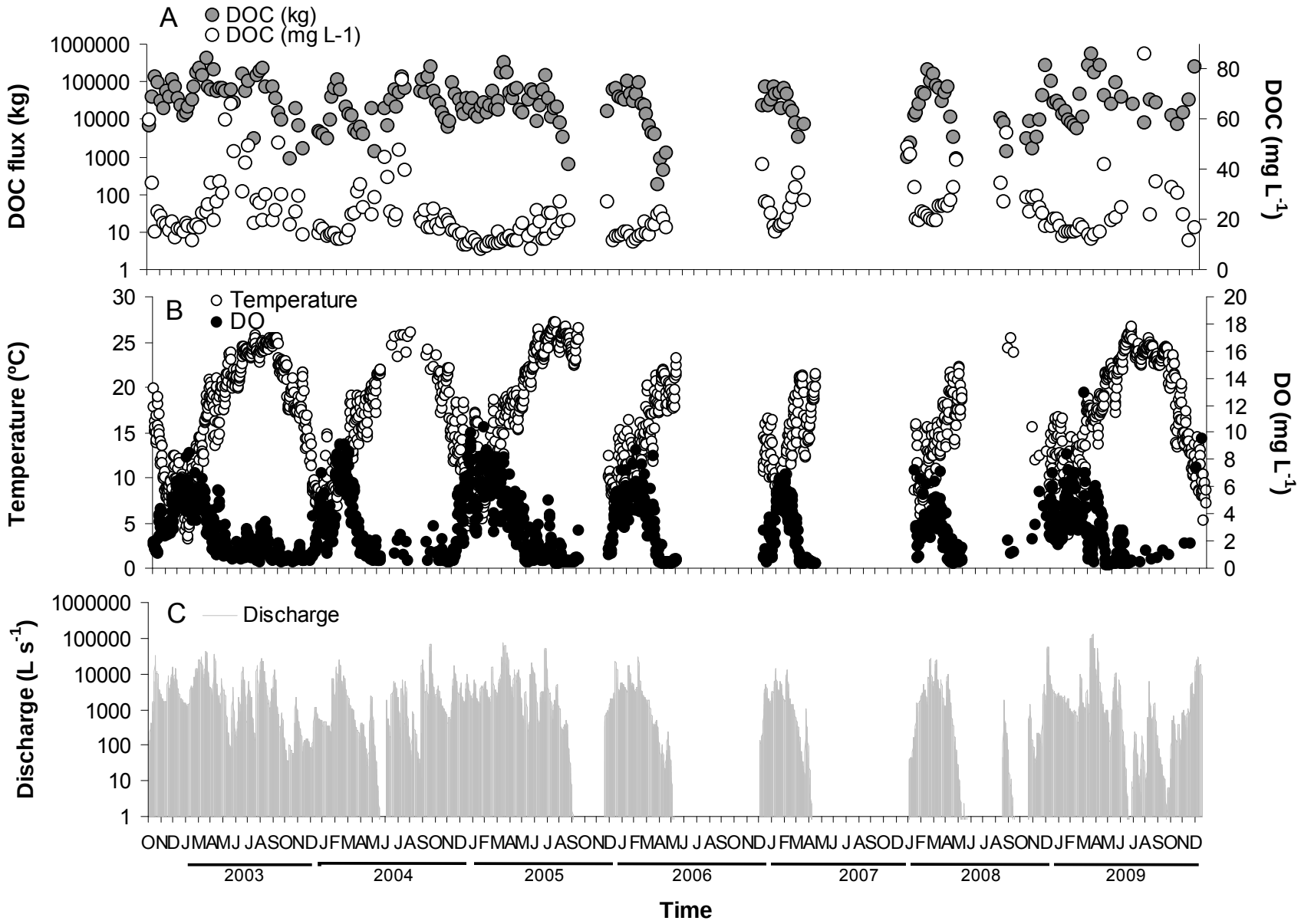

A
DOC (kg)
DOC (mg L-1)
DOC flux (kg)
DOC (mg L-1)
B
Temperature
DO
Temperature (ºC)
DO (mg L-1)
C
Discharge
Discharge (L s-1)
ONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASONDJFMAMJJASODJFMAMJJASONDJFMAMJJASONDJFMAMJJASOND
2003
2004
2005
2006
2007
2008
2009
Time

Figure 5.3: DOC concentration (mg L^{-1}) regressed against discharge (L s^{-1}, logarithmic scale) from 2003-2009.

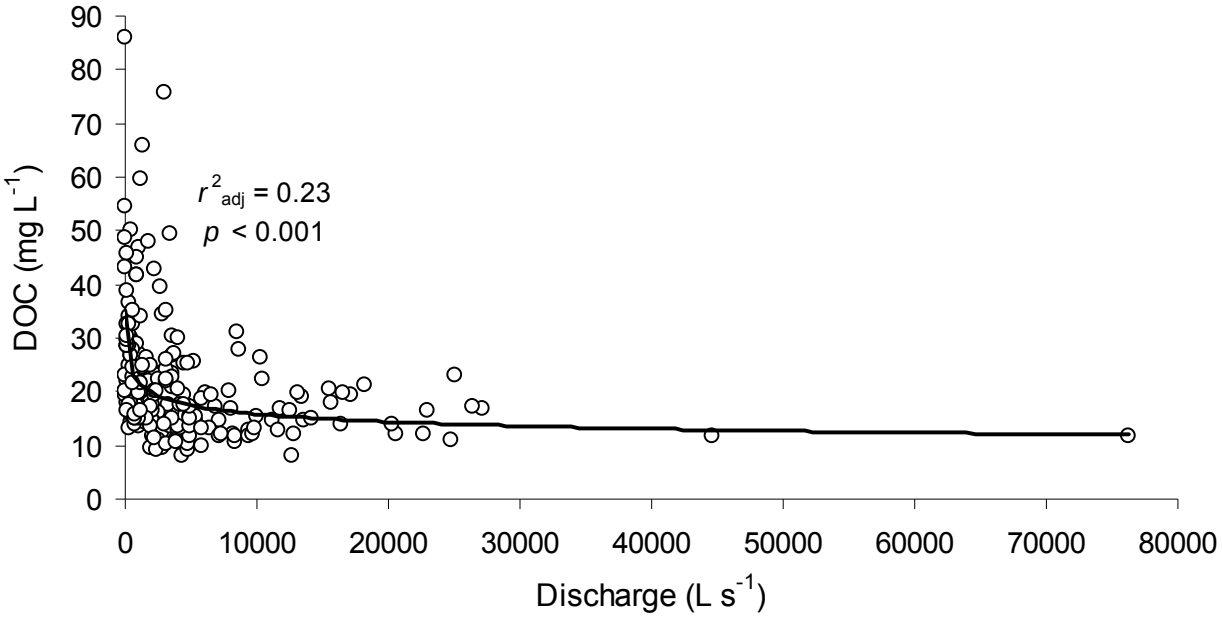
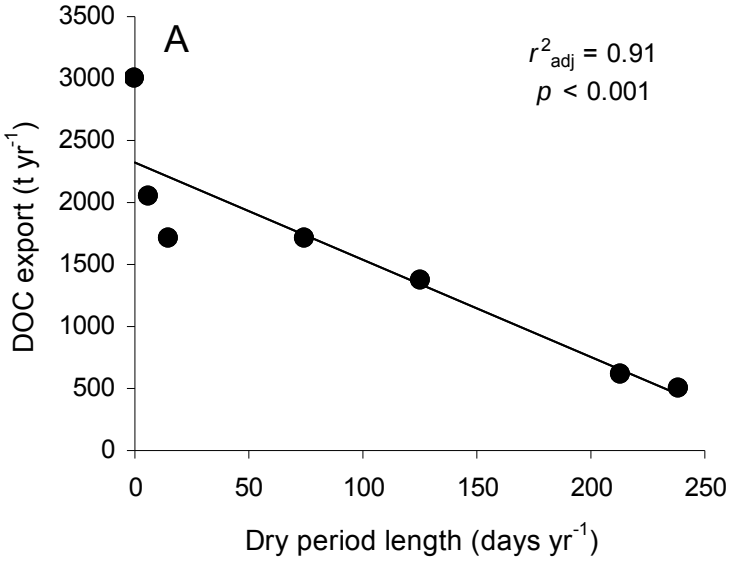

90
80
70
60
50
40
30
20
10
0
DOC (mg L-1)
r2adj = 0.23
p < 0.001
0
10000
20000
30000
40000
50000
60000
70000
80000
Discharge (L s-1)

Figure 5.4: Dry period length, DOC export, and effects on DOC concentration. A) DOC export (metric tons $year^{-1}$) regressed against dry period length (days) from 2003-2009. B) Average (black circles) and minimum (white circles) DOC concentration (mg L^{-1}) in the current hydroperiod regressed against export (metric tons) in the previous hydroperiod.


A
r^2_{adj} = 0.91
p < 0.001
DOC export (t yr^{-1})
Dry period length (days yr^{-1})
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
250

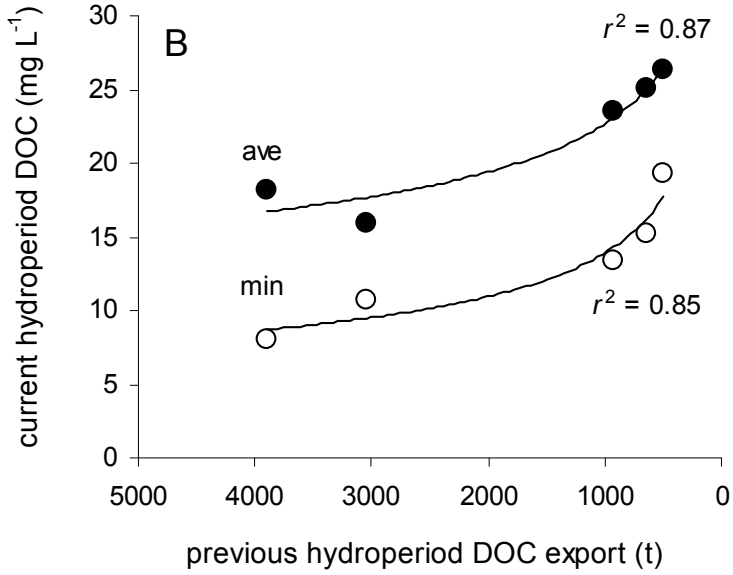
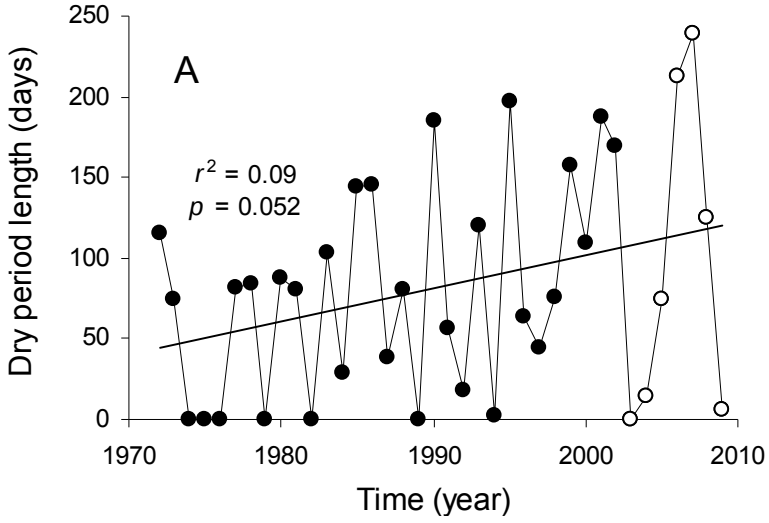

B
r^2 = 0.87
ave
min
r^2 = 0.85
current hydroperiod DOC (mg L^{-1})
previous hydroperiod DOC export (t)
30
25
20
15
10
5
0
5000
4000
3000
2000
1000
0

Figure 5.5: Little River Experimental Watershed annual dry period length and rainfall from 1972-2009. A) Annual dry period length over time, with years of the study period indicated by open symbols and B) maximum annual dry period length (gray columns, days) and minimum annual rainfall (white circles, mm) per 5-year-increment from 1972 and 2009.


A
r^2 = 0.09
p = 0.052
Dry period length (days)
Time (year)
250
200
150
100
50
0
1970
1980
1990
2000
2010

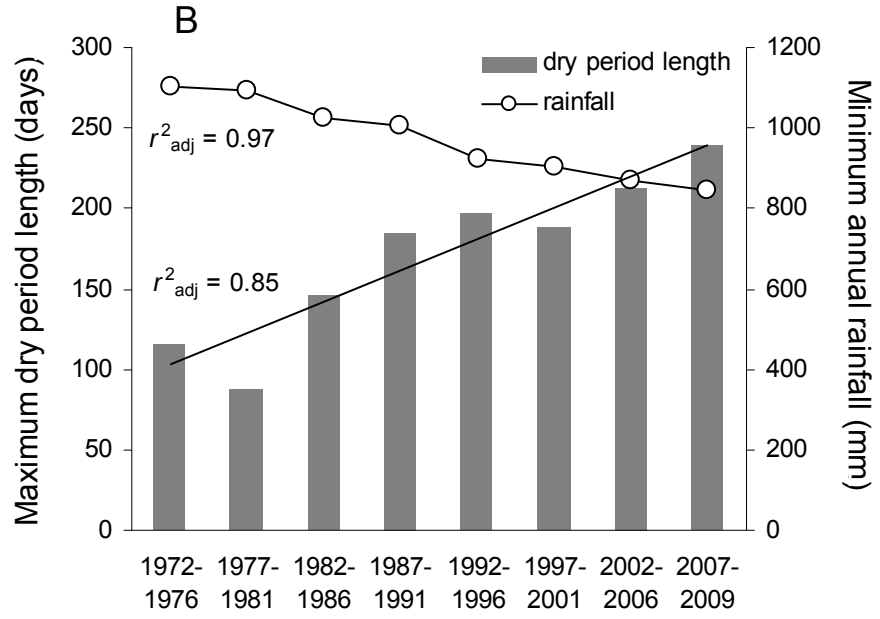

B
dry period length
rainfall
r^2_{adj} = 0.97
r^2_{adj} = 0.85
Maximum dry period length (days)
Minimum annual rainfall (mm)
300
250
200
150
100
50
0
1200
1000
800
600
400
200
0
1972-1976
1977-1981
1982-1986
1987-1991
1992-1996
1997-2001
2002-2006
2007-2009

Figure 5.6: Fluorescence signatures of the three PARAFAC components identified in the LREW dataset. Contour plots of components C1-C3 are ordered by decreasing percent explained. Corresponding line plots to the right of each contour plot show each component's excitation (left, black) and emission (right, gray) spectra. See Table 4 for component descriptions.

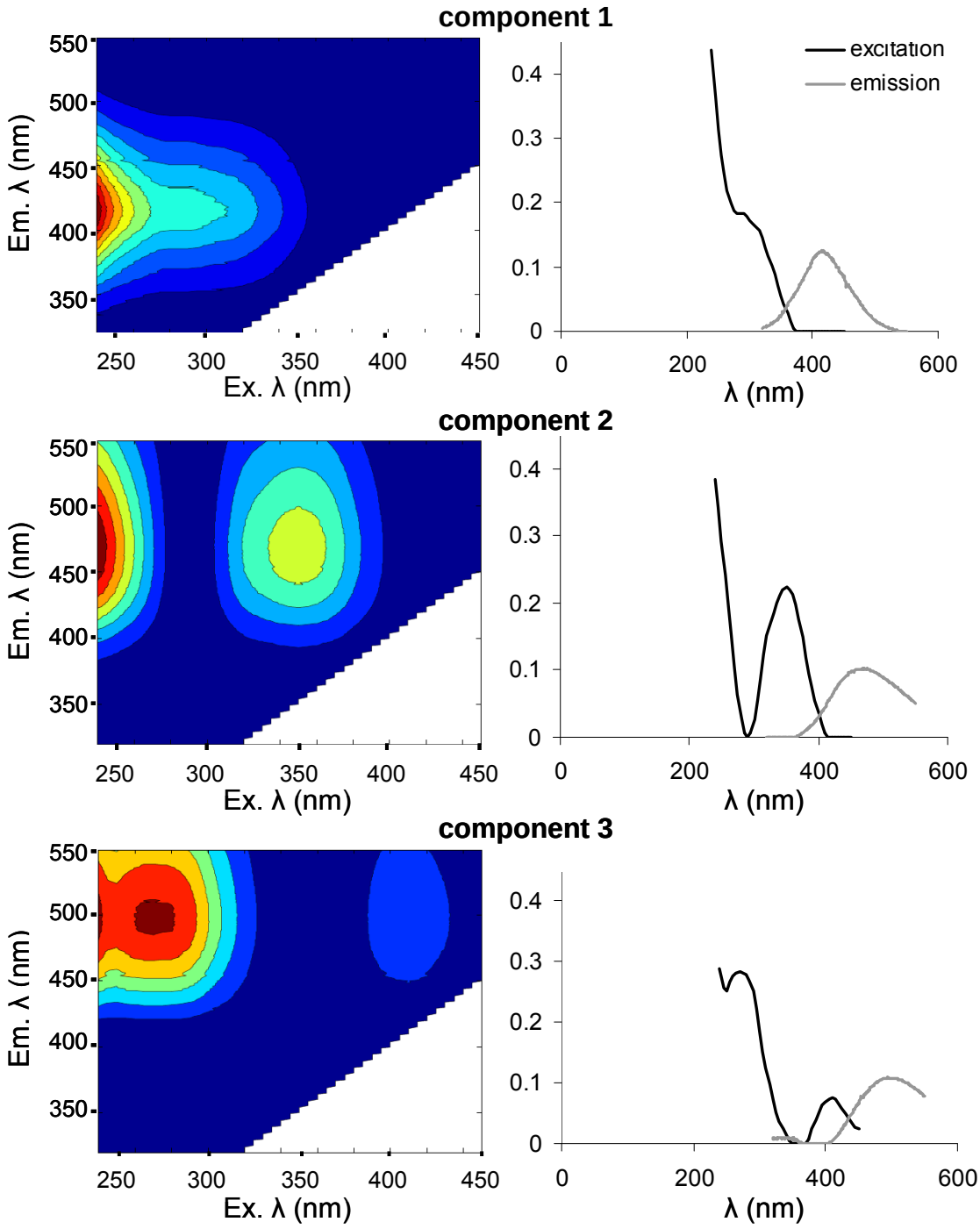
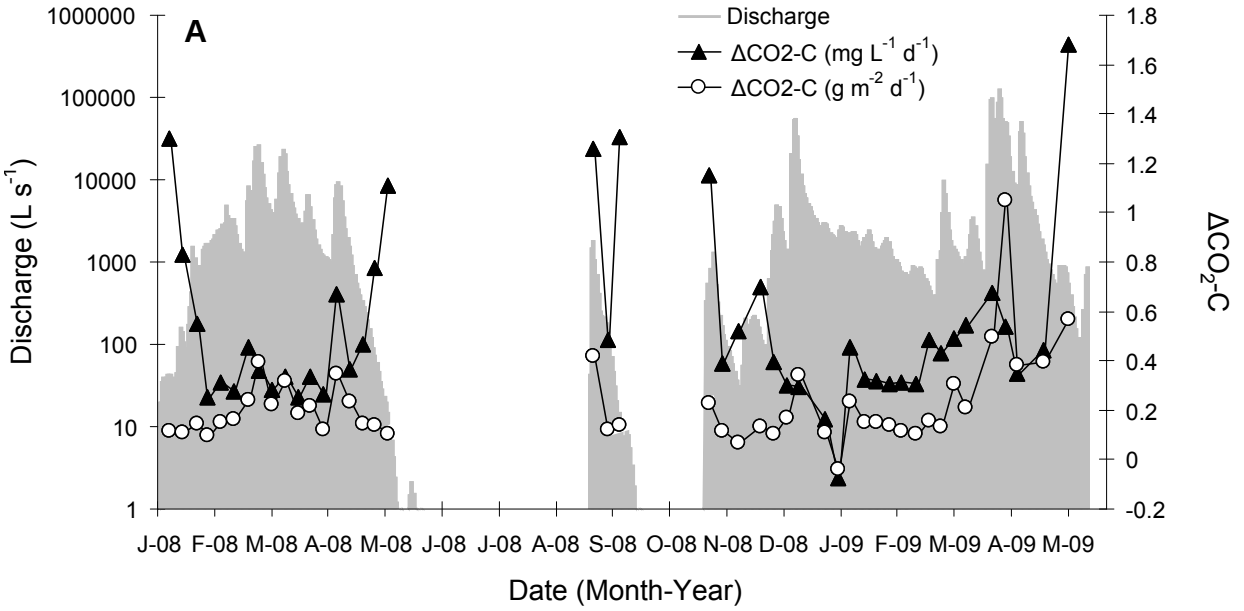

component 1
Em. λ (nm)
Ex. λ (nm)
λ (nm)
excitation
emission
component 2
Em. λ (nm)
Ex. λ (nm)
λ (nm)
component 3
Em. λ (nm)
Ex. λ (nm)
λ (nm)

Figure 5.7: DOC mineralization rates over time, and DOC mineralization regressed against DOC concentration. A) DOC mineralization rate expressed per liter (mg CO_2-C L^{-1} day^{-1}, black triangles) and on an areal basis (g CO_2-C m^2 day^{-1}, white circles). Discharge (L s^{-1}) is shown in logarithmic scale. B) ΔDOC (white symbols, dashed line) and ΔCO_2-C (black symbols, solid line) (mg C L^{-1} day^{-1}) regressed against DOC concentration (mg L^{-1}).


A
Discharge
ΔCO2-C (mg L-1 d-1)
ΔCO2-C (g m-2 d-1)
Discharge (L s-1)
ΔCO2-C
1000000
100000
10000
1000
100
10
1
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
J-08 F-08 M-08 A-08 M-08 J-08 J-08 A-08 S-08 O-08 N-08 D-08 J-09 F-09 M-09 A-09 M-09
Date (Month-Year)

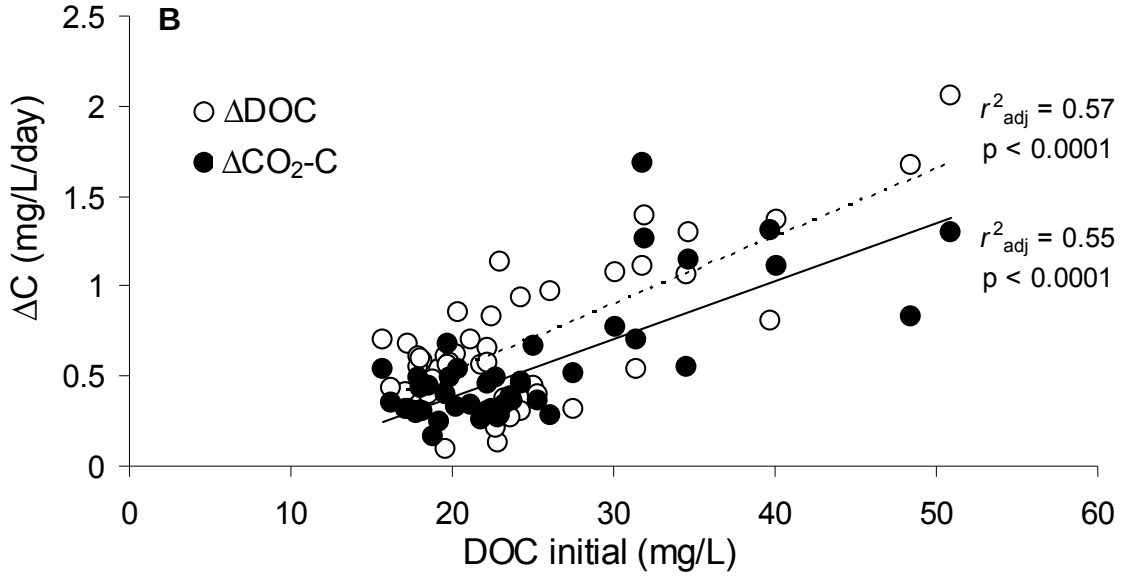
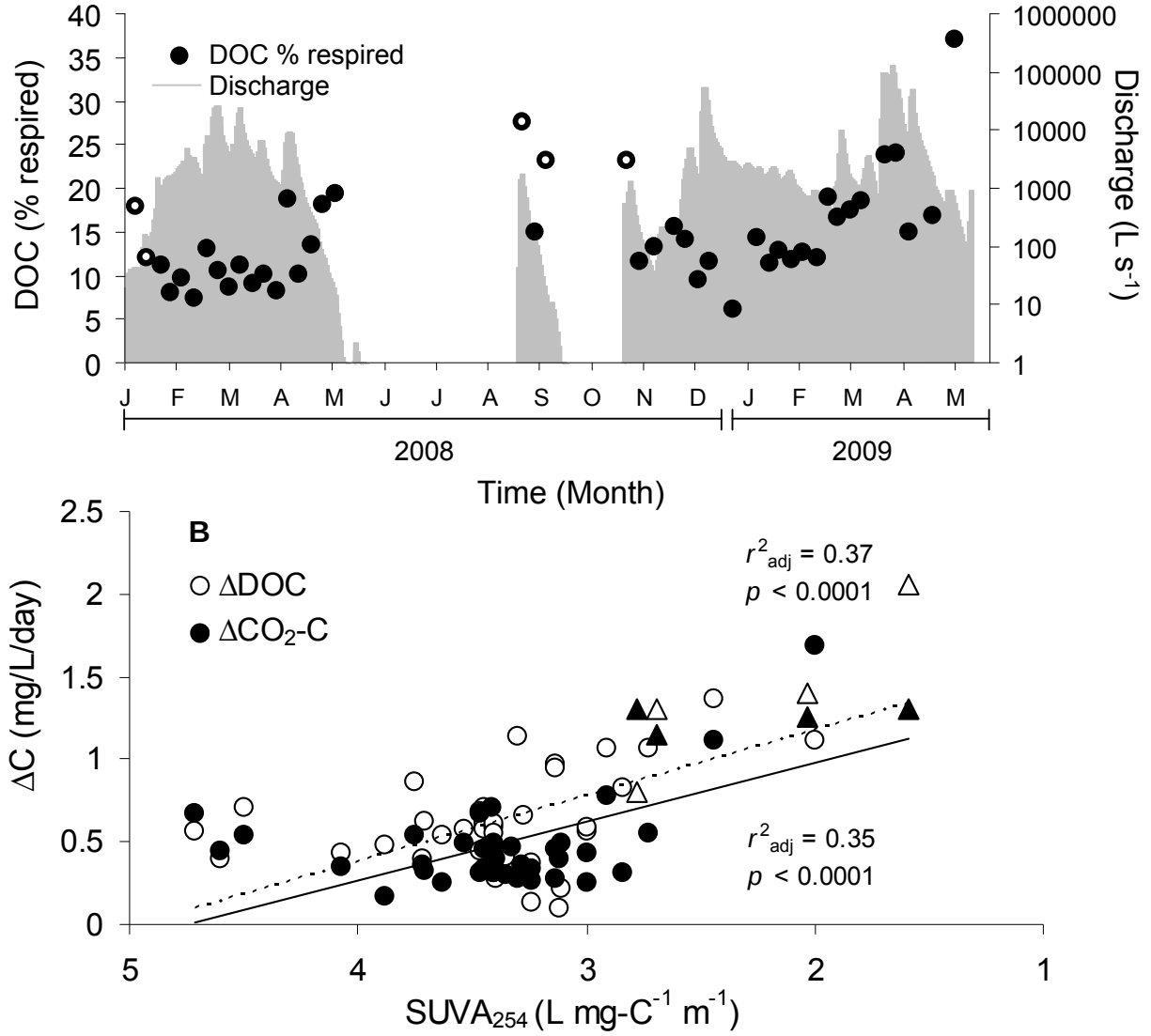

B
ΔDOC
ΔCO2-C
r2adj = 0.57
p < 0.0001
r2adj = 0.55
p < 0.0001
ΔC (mg/L/day)
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
DOC initial (mg/L)

Figure 5.8: Temporal changes in DOC bioavailability, and DOC mineralization rate regressed against $SUVA_{254}$. A) DOC bioavailability (percent respired) over time (months) in 2008 and 2009, with discharge ($L\ s^{-1}$) in logarithmic scale. Black symbols with white central points indicate outliers which were not included in PARAFAC models. B) ΔDOC (white symbols, dashed line) and ΔCO_2-C (black symbols, solid line) ($mg\ C\ L^{-1}\ day^{-1}$) regressed against $SUVA_{254}$, with triangles indicating outliers for which DOM components were not modeled. Four of the five outliers occurred during wet-dry transition.


DOC % respired
Discharge
DOC (% respired)
Discharge (L s-1)
J F M A M J J A S O N D J F M A M
2008
2009
Time (Month)
B
ΔDOC
ΔCO2-C
r2adj = 0.37
p < 0.0001
r2adj = 0.35
p < 0.0001
ΔC (mg/L/day)
SUVA254 (L mg-C-1 m-1)

CHAPTER 6

GENERAL CONCLUSIONS AND SUMMARY OF THE FINDINGS OF THE LOW DISSOLVED OXYGEN PROJECT

Many blackwater streams and rivers of North America exhibit seasonally low dissolved oxygen, potentially stemming from microbial oxygen uptake during organic matter breakdown (e.g., leaf litter and dissolved organic carbon). The work presented here has illustrated several mechanisms by which leaf litter may impact dissolved oxygen concentrations within blackwater streams and rivers, but also suggest potential effects of forest composition on dissolved oxygen dynamics. Red maple (*Acer rubrum*), water oak (*Quercus nigra*), Ogeechee tupelo (*Nyssa ogeche*), swamp tupelo (*N. biflora*), bald cypress (*T. distichum*), and pine (*Pinus* sp.) produced leaf litter differing significantly in initial content of nutrients and recalcitrant structural compounds, and subsequently differing in microbial biomass and oxygen uptake rates. However, benthic oxygen demand depends not only on the degree to which leaf litter species enhance microbial activity, but also on the amount of leaf litter present in the system, which depends on inputs from riparian and riverine forests, hydrological differences among river reaches, and the rate of leaf litter breakdown. Leaf litter breakdown rates (k) differed among tree species and also between stream and swamp reaches within the Little River. Furthermore, our studies suggest that certain tree species (*T. distichum*) produce more leaf litter per individual tree when compared to other tree species, thus suggesting that forest composition may be able affect the amount of litter (and also oxygen demand) within a river. This may have been due to greater physiological

adaptation to low dissolved oxygen (DO) concentration and perhaps to high concentrations of manganese in the water column.

Estimates of leaf litter oxygen demand within the Little River compared well with prior published estimates of total sediment oxygen demand (SOD) measured either directly or estimated through modeling, These finding highlight the importance of leaf litter to oxygen dynamics within the system, but discrepancies between maximum values of leaf litter oxygen demand and SOD suggest that additional mechanisms of oxygen demand exist. Dissolved organic carbon (DOC) leaching from leaf litter may also affect microbial respiration, but while we identified the likely drivers of large fluctuations of DOC concentration in the Little River, the total oxygen demand generated by microbial utilization of DOC is likely small compared to that generated by leaf-litter-associated microorganisms. These findings compliment a large body of work previously conducted by other researchers studying dissolved oxygen dynamics within the Little River.

Microbial biomass and metal content were both important parameters explaining leaf litter nutrient content (see Chapter 2). Results indicate that while allowing for a great deal of flexibility in leaf litter nutrient leaching rates and concentration of N and P in microbial tissues, a portion of detrital nutrients were not be easily accounted for biotically. Abiotic pools, such as nutrients bound to sediments on litter surfaces, may have a impact on bulk detrital nutrient content. Differences in nutrient content persisted over time, suggesting the potential for long-term effects of forest composition on nutrient sequestration by leaf litter. Furthermore, coupled with the very large standing stocks of leaf litter in the swamp (see Chapter 4), there is potential for substantial removal and sequestration of nutrients from the water column, demonstrating that leaf litter could form a long-term sink for nutrients Agricultural impacts are

not readily apparent within the Little River, because water column nutrient concentrations are relatively low for an agricultural watershed (Feyereisen et al. 2008). However, inference of trophic state of a water body from levels of nutrient pollution (P and N) alone may be somewhat misleading, if there is not an accompanying understanding of nutrient loading and uptake rates within the system (Dodds 2003). It has previously been reported that denitrification was a large source of DIN removal in riparian zones within the Little River watershed (Vellidis et al. 2003a), and a large quantity of N may be exported from the system via this dissimilatory microbial pathway. However, as we have established significant N sequestration by leaf litter microbes (Chapter 2), and SOD rate displays a significant positive correlation to C and total N content in organic-matter-rich sediments (Todd et al. 2009), there is a direct link between benthic oxygen demand and nutrient sequestration by detritus.

Through geostatistical modeling, Todd et al. (2010) previously demonstrated that SOD rates could be predicted by concentrations of total organic carbon (including detritus) within sediments of the Little River, suggesting the importance of leaf litter and other forms of detritus to dissolved oxygen dynamics within blackwater rivers. Leaf-litter-generated oxygen demand in blackwater swamps and streams is controlled by a complex interaction between leaf litter chemistry, microorganisms, and larger consumers that facilitate the breakdown of organic matter and its export from aquatic ecosystems (Baxter et al. 2005, Gratton and Zanden 2009). We showed that leaf litter species with lowest initial concentrations of lignin, such as *N. ogeche*, supported highest microbial biomass and respiration, providing a mechanistic link between forest composition and leaf-litter-associated oxygen demand (see Chapter 3). Microbial biomass changed significantly over time and gradually declined as the lignocellulose index (LCI) of litter increased, indicating that leaf litter species effects on microbial processes also changed over time

in ways similar to those conceptually outlined by (Moore et al. 2004). As expected, more rapid breakdown was observed on labile plant litter in the presence of high macroinvertebrate shredder biomass, suggesting that recalcitrant litter has the potential for greater long-lasting ecosystem effects on metabolism, by way of sheer resilience. Furthermore, because macroinvertebrate biomass is twice as high in the swamp than in the stream, breakdown rates of labile litter were also twice as high. Leaf litter breakdown rate is an important parameter used in models designed to predict blackwater river DO concentrations, but up until now most data for model parameterization were taken from Meyer et al. (1997). The differences in leaf litter breakdown rates measured in two sites within the Little River casts doubt on the validity of assuming that leaf litter breakdown rates from the Ogeechee River will accurately represent those in the Little River.

Leaf litter inputs, standing stocks, and litter-associated microbial biomass and respiration were measured in stream and swamp reaches within the Little River (see Chapter 4). Total annual litter inputs to the swamp averaged 995 g m^{-2} y^{-1}, which is high among litterfall estimates in cypress-dominated aquatic ecosystems (Middleton and McKee 2004). Pond cypress was significantly more dominant in the deepest portions of the swamp, and its litter inputs per total trunk biomass per area (g dbh^{-1} m^{-2} y^{-1}) were significantly higher than those of swamp tupelo and Ogeechee tupelo, possibly reflecting adaptations to low DO and elevated manganese concentrations that are observed within the site. Less leaf litter was present in the stream, due both to lower inputs compared to the swamp, and also because of greater decreases in leaf litter standing stock in the stream than in the swamp. Leaf litter breakdown rates in the stream are much lower than those in the swamp, and so it is more likely that higher water velocity within the stream, based on estimates by (Cathey 2005), exported more leaf litter. Through the used of

rhodamine dye as a tracer, Todd (2008) also measured higher water velocities and shorter water residence times in the stream compared to the swamp, and suggested that longer water residence times may have resulted in greater depletion of DO in the swamp, due to increased time for oxygen uptake. Our results suggest an additional linkage between water residence time and DO dynamics: As residence time increases and velocity decreases, the retentiveness of the system is enhanced, thereby flushing less oxygen-consuming organic matter out of the system. More than half of the leaf litter standing stocks present at the beginning of a wet period remained in the swamp long after the transition from wet to dry phases, suggesting accumulation of organic matter over time.

In a detailed examination of the parameterization and validation of a model (DoSag) predicting DO concentration within the Little River, Cathey (2005) identified reaeration and SOD to be the two most sensitive parameters. Subsequently, Todd et al. (2009) measured SOD directly in two reaches of the Little River, and observed extremely high SOD rates within an instream swamp. Significant positive correlation between SOD and concentrations of organic C and total N in sediments highlighted the potential importance of detritus in SOD in their study. Mean estimates of leaf litter standing stock respiration in the current study (5.54 g O_2 m^{-2} day^{-1}, see Chapter 4) accounted for 89% of mean SOD directly measured by Todd et al. (2009), and were only slightly lower than calibration values (6 g O_2 m^{-2} day^{-1}) required for validation of DoSag models in the Little River (Cathey 2005). However, the highest SOD measurements reported by Todd et al. (2009) were greater than 14 g O_2 m^{-2} day^{-1}, while highest leaf litter standing stock respiration estimates barely exceeded 8 g O_2 m^{-2} day^{-1}.

These differences may be explained by additional unmeasured oxygen sinks within the Little River. Respiration by animals has been shown to substantially contribute to SOD in other

studies (Butts and Evans 1978), and leaf pack macroinvertebrate biomass was twice as high in the swamp than in the stream reach in the current study, so animal respiration also may explain a portion of the oxygen demand not accounted for by leaf-litter-associated microbial respiration. Potential respiration of woody debris could not account for the disparity, but large quantities of dead filamentous diatoms and algae were encountered in standing stock samples collected late in the wet season, and they may generate a large additional oxygen sink within the Little River if they were to decompose in the benthos.

Carey et al. (2007) used nutrient diffusing substrates (NDS) to assess nutrient and light limitation of algal growth within nine blackwater stream sites within Georgia's coastal plain, and concluded that algal growth was primarily light limited and secondarily nutrient limited. However, this does not agree with substantial filamentous diatom (*Fragilaria* sp.) and algal (*Microspora* sp.) growth being observed by several researchers' (G. Vellidis, K. A. Kuehn, M. J. Todd, personal communication 2008) the Little River watershed, as well as their collection in leaf litter standing stock samples in the current study (see chapter 4). Carey et al. (2007) deployed NDS between April and June, well into the spring when canopies are relatively filled in. Although light limitation had significant effects on periphyton growth at that time, the study was conducted toward the end of the wet season, when tree canopies may have been full enough to substantially reduce light penetration. These months in which water is present and trees have not leafed out may provide better conditions for algal growth, and further assessment of seasonal changes in algal standing crop may better resolve the role of periphyton in DO dynamics within the Little River.

Both precipitation and stream flow have gradually declined in the Little River Experimental Watershed since water quality monitoring began (Feyereisen et al. 2008), and

rainfall is expected to continue to decrease throughout the region (Mearns et al. 2003), accompanied by increased drought intensity (Dai 2011). If these trends continue, this may ultimately pose one of the greatest challenges facing watershed management and policy in Georgia's coastal plain, with potentially large effects on DO dynamics and carbon cycling in blackwater rivers. Todd (2008) suggested that increased water residence times may reduce DO concentration in riverine swamps through increased contact time between water and underlying sediments and organic matter. If stream flow is curtailed in decades to come, this effects may be potentially enhanced. In the current study, seven years of data indicated that annual DOC transport decreases approximately eight metric tons for each one-day increase in drought length (see Chapter 5). Furthermore, after years with short hydroperiods, resulting in reduced DOC exports, DOC concentration in the following year was significantly higher. DOC concentration within hydroperiods also increased at high temperature and low discharge. DOC utilization experiments indicated that bacteria consume more DOC and release more CO_2 when DOC concentrations are high. These findings suggest that as droughts intensify, temperatures rise, and discharge decreases, river carbon cycling will change, with increased CO_2 export via increased DOC concentrations, and reduced DOC transport downstream to coastal areas.

Georgia's new region-specific water quality criteria are a fairly progressive step toward a new statewide water policy that does a better job of incorporating biological processes, while recognizing regional differences in natural environmental conditions (i.e. gradient, temperature). To date, the Georgia Department of Natural Resources-Environmental Protection Division (GADNR-EPD) has removed most of the streams that were originally included on the 303d list as being cited for DO impairment. Many of these decisions are based on water column nutrient concentrations that do not appear high enough to suggest excessive anthropogenic nutrient

loading. However, the work presented here suggests that new approaches to water management may be necessary when DO is being considered. Inference of trophic status of a water body from levels of water column nutrient concentration may be somewhat misleading, if there is not an accompanying understanding of nutrient loading and uptake rates within the system (Dodds 2003). For example, in chapter 1 we showed that leaf litter may sequester large quantities of nutrients from the water column. These nutrients may be incorporated directly into microbial biomass, and may enhance oxygen consumption by aquatic fungi and bacteria. Nutrients taken up and stored in leaf litter or sediments may not be readily detected in the water column, nor would they result in algal blooms if there was sufficient light limitation, but they could still contribute substantially to oxygen demand through enhancement of benthic microbial biomass.

The greatest future challenge for Georgia land managers who monitor DO levels may be water availability. Maintaining adequate river flow is crucial to keeping DO concentrations within an acceptable range, because higher water velocity increases turbulence, thereby enhancing reaeration. In addition, water can export organic matter downstream, thereby lowering carbonaceous oxygen demand within a reach. If rainfall continues to decrease over time, meeting irrigation demands while maintaining the river flows necessary to uphold DO standards may become increasingly more difficult. A better understanding of the effects of the numerous farm ponds scattered throughout the landscape on flow will be needed if rainfall throughout southern Georgia continues to decline.

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APPENDIX A

Inorganic constituents of leaf litter and from Tifton soils of the Georgia coastal plain. Mean leaf litter inorganic constituents are expressed as a percentage of total inorganic matter per gram of litter (range in parentheses, n = 3). Inorganic constituents from Tifton soils* of the Georgia coastal plain are expressed as percent total soil dry weight. *Data from Schumacher 1982.

Little River, leaf litter inorganic matter

days	Mg	K	Ca	Si	Al	Fe	Mn	Na	clay	silt	sand
0	--	--	--	--	0.63 (0.38-0.90)	0.65 (0.57-0.81)	3.31 (1.61-4.20)	--	--	--	--
36	4.36 (1.23-9.27)	2.73 (0.98-5.94)	26.35 (8.28-55.99)	7.30 (5.32-10.52)	1.50 (1.07-1.93)	3.19 (2.89-3.76)	1.26 (0.56-1.93)	trace	--	--	--
173	1.63 (0.46-3.63)	1.07 (0.53-2.03)	12.73 (3.60-29.39)	11.72 (9.75-13.65)	4.09 (3.34-4.59)	6.98 (5.87-8.24)	2.01 (1.09-3.50)	trace	--	--	--
431	1.20 (0.58-1.81)	0.91 (0.64-1.09)	7.53 (3.08-11.65)	10.89 (9.89-12.62)	4.47 (3.98-5.04)	9.60 (7.32-12.71)	3.64 (3.17-4.32)	trace	--	--	--

Tifton soil

Horizon (depth in cm)	Mg	K	Ca	Si	Al	Fe	Mn	Na	clay	silt	sand
Upper B (81-107)	7.44	3.11	4.80	25.0	10.3	3.7	--	1.61	19.4	4.2	76.5
Middle B (107-132)	6.06	4.31	3.60	22.5	13.3	5.4	--	3.40	22.8	3.6	73.7
Lower B (132-152)	5.53	2.63	3.20	21.1	13.2	7.5	--	4.39	30.0	2.4	69.5
BC (152-183)	5.09	2.89	2.60	18.1	12.4	9.9	--	1.61	43.3	2.6	54.1
C (>183)	4.84	2.83	2.00	23.2	9.8	8.1	--	2.81	56.2	4.5	39.3

APPENDIX B

Calculations and literature sources used in the development of predicted detrital nutrient models.

$N_{estimated}$:

$$N_{bacterial} + N_{fungal} + N_{leached\ litter}$$

$P_{estimated}$:

$$P_{bacterial} + P_{fungal} + P_{leached\ litter}$$

$N_{bacterial}$:

$$\frac{AFDM_t \times C_{bacterial,t}}{C{:}N_{bacterial}}$$

Where:

$AFDM_t$ = leaf litter ash-free dry mass (g) after incubation period (t = time, in days)

$C_{bacterial,t}$ = bacterial carbon (mg g^{-1}) after incubation period (t = time, in days)

$C{:}N_{bacterial}$ = bacterial C:N ratio, ranging from 2.62-17.1 (Bratbak 1985, Lee and Fuhrman 1987, Tezuka 1990, Chrzanowski and Kyle 1996, Chrzanowski et al. 1996, Fagerbakke et al. 1996, Vrede et al. 2002, Makino and Cotner 2004, Thingstad et al. 2005)

$P_{bacterial}$:

$$\frac{AFDM_t \times C_{bacterial,t}}{C{:}P_{bacterial}}$$

Where:

$AFDM_t$ = leaf litter ash-free dry mass (g) after incubation period (t = time, in days)

$C_{bacterial,t}$ = bacterial carbon (mg g^{-1}) after incubation period (t = time, in days)

$C{:}P_{bacterial}$ = bacterial C:P ratio, ranging from 8-260 (Bratbak 1985, Jürgens and Güde 1990, Tezuka 1990, Chrzanowski and Kyle 1996, Chrzanowski et al. 1996, Fagerbakke et al. 1996, Hochstädter 2000, Vrede et al. 2002, Makino et al. 2003, Makino and Cotner 2004, Thingstad et al. 2005)

C_{fungal}:

$$AFDM_t \times ergo_t \times \frac{1}{d.m.c.f.} \times d.m.{:}C$$

Where:
$AFDM_t$ = leaf litter ash-free dry mass (g) after incubation period (t = time, in days)
Appendix B continued:

$ergo_t$ = ergosterol content (μg g^{-1} leaf litter dry mass) after incubation period (t = time, in days)

d.m.c.f. = ergosterol to dry mass conversion factor, ranging 2.3-11.5 μg ergosterol / mg fungal dry mass (Gessner and Chauvet 1993)

d.m.:C = dry mass to C conversion factor, ranging from .420-.447 (Baldy and Gessner 1997, Montgomery et al. 2000)

N_{fungal}:

$$\frac{C_{fungal,t}}{C{:}N_{fungal}}$$

Where:
$C_{fungal,\,t}$ = C_{fungal} (mg) after incubation period (t = time, in days)

$C{:}N_{fungal}$ = fungal C:N ratio, ranging from 6.083-16 (Newell & Statzell-Tallman, 1982, Leach & Gulis, 2011, personal communication,)

P_{fungal}:

$$\frac{C_{fungal,t}}{C{:}P_{fungal}}$$

Where:
$C_{fungal,\,t}$ = C_{fungal} (mg) after incubation period (t = time, in days)

$C{:}P_{fungal}$ = fungal C:P ratio, ranging from 40-203 (Leach & Gulis, 2011, personal communication)

$N_{leached\ litter}$:

$$(AFDM_{t=0} \times N_{t=0}) \times (1 - \%N\,loss_{leaching})$$

Where:
$AFDM_{t=0}$ = initial (pre-incubation) leaf litter ash-free dry mass (g)

$N_{t=0}$ = initial (pre-incubation) N content (mg g^{-1})

$\%N\,loss_{leaching}$ = percentage of total leaf litter N lost during 0-10 days of leaching, ranging 0-25% (Melillo et al. 1984, Ibrahima et al. 1995)

$P_{leached\ litter}$:

$$(AFDM_{t=0} \times P_{t=0}) \times (1 - \%P\,loss_{leaching})$$

Where:
$AFDM_{t=0}$ = initial (pre-incubation) leaf litter ash-free dry mass (g)
Appendix B continued:

$P_{t=0}$ = initial (pre-incubation) P content (mg g^{-1})

$\%P\ loss_{leaching}$ = percentage of total leaf litter P lost during 24 hours of leaching, ranging 23.4-39.7% (Meyer 1980, Qiu et al. 2002)

$ADF\%N_{litter}$:

Varying between 0.60-1.05%

non-$ADF\%N_{litter}$:

$([N_{leached\ litter} / AFDM_{t=0}] \times AFDM_t) - (ADF\%N_{litter,\ t=0} \times ADF_t)$
Where:
$AFDM_{t=0}$ = initial (pre-incubation) leaf litter ash-free dry mass (g)
$AFDM_t$ = leaf litter ash-free dry mass (g), after incubation period (t = time, in days)
ADF_t = acid-detergent fiber (g ADF, cellulose + lignin), after incubation period (t = time, in days)

APPENDIX C.

Organisms collected in litter bags in the stream and swamp, with functional feeding group designations. sh = shredder, c-g = collector gatherer, c-f = collector filterer, sc = scraper, pr = predator.

Taxon group	**FFG**	**stream**	**swamp**
Isopoda			
Asellidae			
Lirceus	sh	×	×
Caecidotea	sh	×	×
Amphipoda	sh	×	×
Decapoda			
Cambaridae	sh	×	
Diptera			
Culicidae			
Culex	c-f	×	
Aedes	c-f		×
Chironomidae			
Chironominae			
Rheotanytarsus	c-f	×	
Non-Tanypodinae	c-g	×	×
Tanypodinae	pr	×	×
Ceratopogonidae			
Culicoides	pr		×
Bezzia	pr		×
Tabanidae			
Chrysops	pr	×	
Tipulidae			
Tipula	sh	×	
Dicranota	pr	×	
Simuliidae			
Simulium	c-f	×	
Oligochaeta			
Lumbriculidae	c-g		×
Trichoptera			
Limnephilidae			
Ironoquia	sh	×	×
Pycnopsyche	sh	×	

Appendix C continued:

Taxon group	**FFG**	**stream**	**swamp**
Trichoptera			
Leptoceridae			
Ceraclea	pr		×
Phryganeidae			
Ptilostomis	sh		×
Rhyacophilidae			
Rhyacophila	pr	×	
Polycentropodidae			
Polycentropus	pr	×	×
Bivalvia			
Sphaeriidae			
Sphaerium	c-f	×	×
Gastropoda			
Physidae			
Physella	sc		×
Planorbidae			
Gyraulus	sc	×	×
Freshwater limpet	sc		×
Porifera			
Spongillidae	c-f		×
Turbellaria			
Planariidae			
Planaria	pr		×
Cladocera	sc	×	×
Copedpoda	c-g	×	×
Ostracoda	c-f	×	×
Chilopoda			
Geophilomorpha	pr	×	
Coleoptera			
Hydrophilidae	pr	×	×
Dytiscidae			
Neoporus	pr	×	×
Agabus	pr	×	
Hydaticus	pr	×	
Coptotomus	pr		×
Elmidae			
Stenelmis	sc	×	
unknown	sc		×
Ptilodactylidae			
Anchycteis	sh		×
Collembola	c-g	×	×
Arachnidae			
Acari	pr	×	×
Ephemeroptera			

Appendix C continued:

Taxon group	FFG	stream	swamp
Ephemeroptera			
Leptophlebiidae			
Leptophlebia	c-g	×	
Heptageniidae			
Maccaffertium	sc	×	
Baetidae			
Baetis	c-g	×	×
Amphibia			
Plethodontidae			
Desmognathus	pr	×	
Lepidoptera			
unknown 1	sh	×	
Crambidae	sh	×	
Odonata			
Libellulidae	pr	×	
Aeschnidae	pr		×
Plecoptera			
Perlidae			
Perlesta	pr	×	
Nemouridae			
Ostrocerca	sh	×	
Amphinemura	sh	×	
Perlodidae			
Isoperla	pr	×	
Clioperla	pr	×	×
Nematoda	c-g	×	
Hirudinea	pr		×
Neuroptera			
Sisyridae			
Climacia	pr		×
Heteroptera			
Belostomatidae			
Belostoma	pr		×