THE EFFECTS OF TREE SPECIES ON MICROBIAL RESPIRATION AND LEAF BREAKDOWN IN A COASTAL PLAIN BLACKWATER STREAM

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Abstract. Dissolved oxygen (DO) levels in the Little River, a tributary within the Suwannee River basin of southern Georgia, regularly drop below 1 mg L^{-1}. A budget of coarse particulate organic matter (CPOM) dynamics and the oxygen demand generated by associated microbial respiration is being developed for third- and fifth-order reaches of the Little River. Our CPOM budget will include riparian inputs, standing stocks of various CPOM pools (e.g. leaf litter, woody debris), breakdown, and transport and settling of CPOM and the fine particulate organic matter that results from its processing. For all pools of CPOM, microbial respiration rates are measured over time to assess the oxygen demand generated by these stocks of organic matter. Preliminary research indicates that among tree species, respiration rates of attached microbes differ significantly, and all CPOM inputs and standing stocks will be accordingly sub-divided by tree species in our final budget. Once developed, this budget may provide a reference for policy makers, and a better understanding of the ways biota affect and are affected by oxygen dynamics in coastal plain blackwater streams.

INTRODUCTION AND BACKGROUND

The Suwannee River is still considered relatively undeveloped, and has had no major impoundments constructed within its drainage (Katz and Raabe 2005). However, water quality within the Suwannee River basin has come under question, especially with regards to dissolved oxygen (DO) concentrations. For example, DO concentrations in the Little River, which is included in the Suwannee River basin, have regularly reached levels below 1 mg l^{-1} during the summer months (Joyce et al 1985, Bosch et al 2002).

Although low DO is a common feature of coastal plain blackwater rivers (Meyer 1992), 61 of the 67 water body segments listed on the 2001 303(d) list in the Coastal Plain of Georgia violate standards for DO established by the Georgia Department of Natural Resources Environmental Protection Division (DNR-EPD) (Vellidis et al 2003). This requires the development of total maximum daily load (TMDL) management and implementation plans for watersheds drained by these water body segments. While it is known that DO concentrations below government standards exist within the Suwannee River basin, the factors influencing the oxygen demand are not fully understood. The Georgia DNR-EPD has proposed a new DO standard of 90% of natural DO during critical flow conditions, but little information is available to establish what natural DO concentrations should be (Vellidis et al 2003). Policy development could be enhanced by a better understanding of the factors affecting the observed oxygen demand.

It has been hypothesized that, among others things, a combination of high temperatures and high loads of organic carbon from riparian vegetation contribute to naturally low DO levels in coastal plain streams with otherwise good water quality (Vellidis et al 2003). Microbial respiration rates are naturally high in coastal plain rivers due to the availability of large quantities of organic matter (Meyer 1992), and high inputs of allochthonous leaf litter within coastal plain streams and rivers (Cuffney and Wallace 1987) may support large populations of heterotrophic organisms. The naturally-low DO concentrations of these rivers may make them even more susceptible to nutrient loading (Meyer 1992), as a system already oxygen-limited for natural reasons may not be able to accommodate anthropogenic enhancement of oxygen demand. Inputs of nutrients can significantly enhance the respiration of aquatic microbes (Mallin et al 2004), without producing obvious signs of eutrophication such as algal blooms.

A baseline understanding of natural levels of oxygen demand would be helpful in order to understand ecosystem-level effects of nutrient loading, but oxygen removal due to microbial respiration under natural conditions has not been sufficiently quantified in the Georgia portion of the Suwannee River basin. To gain a better understanding of the system-specific causes of low DO and to provide a reference for policy makers, a budget of coarse particulate organic matter (CPOM) dynamics and the oxygen demand generated by associated microbial respiration is being developed for 3rd- and 5th-order reaches of the Little River. Although land use for the watershed is primarily agricultural, neither reach displays clear evidence of nutrient enrichment. By quantifying the oxygen demand generated by microbes associated with CPOM under known concentrations of nutrients, we may be able to model microbial oxygen demand in other stream reaches.

BUDGET COMPONENTS AND METHODS

For all forms of CPOM included in our proposed budget, associated microbial respiration rates will be measured *in situ* to assess temporal changes in the oxygen demand generated by these stocks of organic matter. Our budget will include riparian inputs, measured with networks of litterfall collectors of a known area. Temporal changes in standing stocks of various pools of CPOM, such as leaf litter (Suberkropp 1997) and woody debris (Benke and Wallace 1990), will be estimated for both reaches. As our respiration estimates apply to CPOM, and not necessarily to the fine particulate organic matter (FPOM) that results from its biological processing, it is necessary to measure leaf breakdown rates (Benfield 1996). Finally, the movement and settling rates of CPOM and FPOM will be estimated through a variety of methods, in order to explain dynamics of organic matter and differences in organic matter accumulations between areas.

Knowing the type (e.g. woody debris, leaf litter) and relative quality (e.g. tree species) of CPOM may be of some importance, rather than measuring masses of organic matter inputs alone. Differences in the quality (represented by chemistry) of CPOM entering streams can have significant effects on aquatic macroinvertebrates (Bärlocher and Kendrick 1973, Kaushik and Hynes 1971) and microbes (Gessner and Chauvet 1994). The results of a preliminary study conducted in 2006 provided information relevant to this topic.

TREE SPECIES EFFECTS - METHODS

The leaves of red maple (*Acer rubrum* var. *trilobum*), Ogeechee tupelo (*Nyssa ogeche*), and water oak (*Quercus nigra*) were collected from the forest floor after abscission and air-dried. Plastic mesh pecan bags (19.1 cm × 38.1 cm, 5 × 5 mm mesh; Cady Industries Inc., Georgia) were filled with leaves (10 grams per bag) of individual tree species, and were attached to pvc arrays using plastic ties. A single array held three bags, each bag containing the leaves of a different tree species, and arrays were grouped into blocks based on location (distance upstream) in the stream channel (Fig. 1). Arrays were anchored to cinder blocks and submerged in a 3rd-order reach of the Little River in Turner County, GA, on February 4th, 2006. Five bags of each species treatment (one from each block) were sampled on a bi-weekly basis, and respiration was measured in situ using 26 ml respiration chambers and a YSI 5100 dissolved oxygen meter (Yellow Springs, OH) that simultaneously recorded DO concentrations (mg l^{-1}) and temperature (ºC). Oxygen uptake was determined by the slope of the regression of DO concentration versus time, minus a control slope using stream water alone.

Samples for fungal (Newell et al 1988) and bacterial (Porter and Feig 1980) biomass were collected and preserved in the field and immediately refrigerated. In the laboratory, leaves were rinsed over nested sieves (250 µm and 1 mm) and macroinvertebrates were preserved in 70% ethanol for future analysis. Leaves were dried for one week at 60ºC, weighed, and a sub-sample was combusted at 500ºC to determine ash-free dry mass (AFDM) and loss of mass over time.

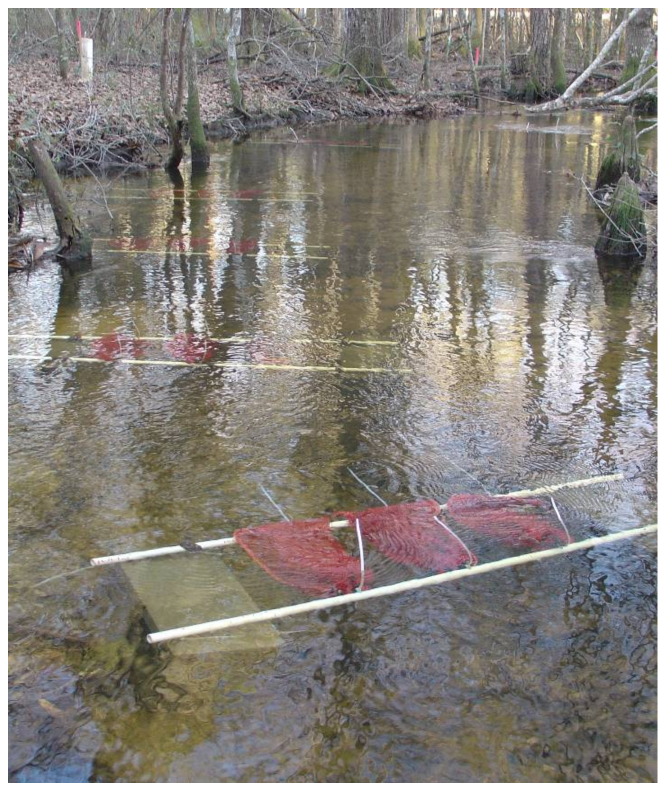

Figure 1. Design of pvc and cinder block arrays of leaf litter bags, submerged in a third-order reach of the Little River, Turner County, GA.

Table 1. Stream data on respiration sampling dates.

Sampling date (2006)	10-Feb	24-Feb	12-Mar	24-Mar	7-Apr
Days of incubation	6	20	36	48	62
Temperature (ºC)	9.5	15.1	21.9	14.5	18.8
pH	5.02	5.32	4.87	4.99	5.27
DO mg l^{-1} (in stream)	10.58	9.27	6.78	8.78	6.06

STATISTICAL ANALYSIS

All statistical analyses except for the generation of confidence intervals were conducted using SAS version 9.1 (SAS Institute Inc., Cary, USA). When necessary, data were transformed to meet assumptions of homogeneity of variances and normality. The effects of tree species, time, and temperature (covariate) on microbial respiration were analyzed by fitting an A×B Factorial Analysis of Covariance (ANCOVA) model with a single blocking factor (location in the stream channel) to the data, and effects of tree species on mass loss were analyzed using a 1-way Analysis of Variance with a single blocking factor (location in the stream channel). Differences between treatment levels for the factors of tree species and time were determined through pair-wise comparisons (Tukey's Honest Significant Difference). Confidence intervals were placed around treatment means using SPSS (SPSS, Inc. 1999).

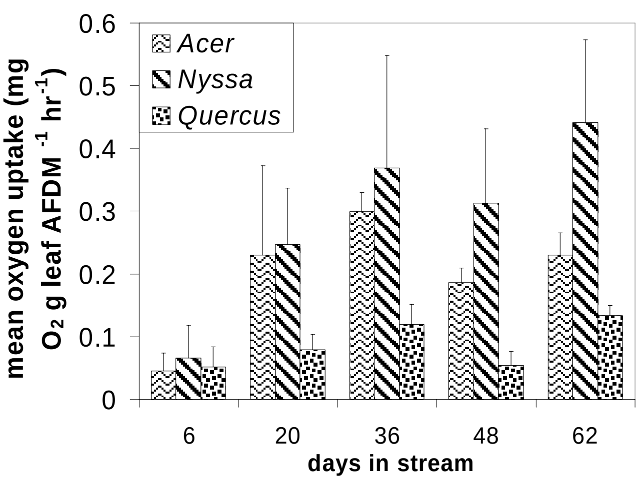

Figure 2. Respiration of leaf litter as affected by time (days in stream) and tree species.

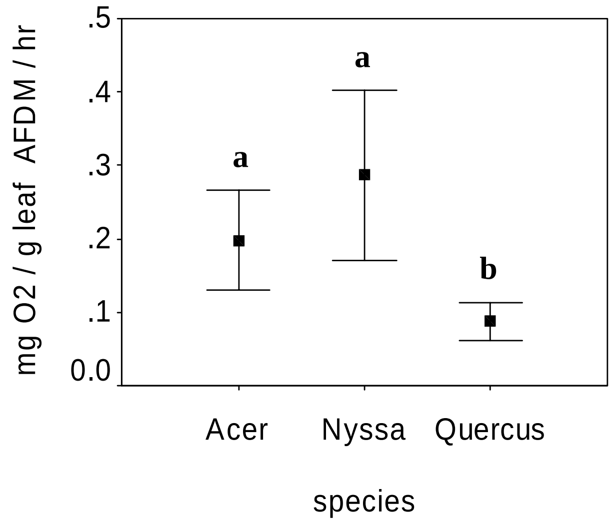

Figure 3. Mean oxygen uptake rates for each tree species, with 95% confidence intervals placed around the treatment mean for each species. Different letters above two means indicate a statistically significant difference between the means (*p* < 0.01).

RESULTS AND DISCUSSION

Respiration. Oxygen uptake rate was affected by differences in tree species ($F_{2,31}$=8.90, *p*=0.0009), days in the stream ($F_{4,31}$=3.55, *p*=0.017), and temperature ($F_{1,31}$=12.22, *p*=0.0014) (Fig. 2). The effects of temperature were particularly noticeable on day 48; all respiration rates were lower than the preceding and following days, presumably due to decreased microbial respiration at colder temperatures (Fig. 2, Table 1). It is also worth noting that on the same day, dissolved oxygen concentration in the stream is higher than on the preceding or following day (Table 1), possibly due to decreased microbial oxygen demand and increased solubility of oxygen in water at lower temperatures. Mean oxygen uptake per gram of *A. rubrum* leaf was not significantly different from that of *N. ogeche* leaves, but both species differed significantly from *Q. nigra* ($p < 0.01$) (Fig. 3). Pair-wise comparisons between numbers of days in the stream also revealed that the treatment effects of different incubation times were significantly different in all comparisons (p<0.05).

Mass loss. Rates of mass loss (grams day^{-1}) differed significantly between all three tree species in pair-wise comparisons (p < 0.001) (Fig. 4). An ANOVA of the total mass lost from leaves sampled on day 73 (the last sampling day before significant dry-down of the stream) also indicated a significant effect of tree species ($F_{2,11}$=161.87, p<0.0001). The percentage of dry mass remaining decreased steadily for all three species of leaves

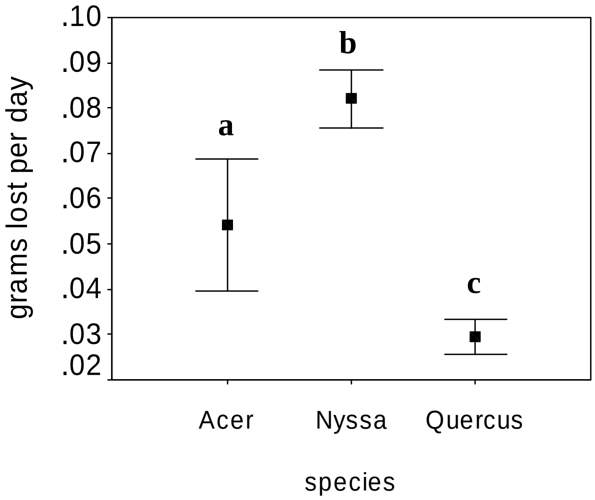

Figure 4. Mean rate of mass loss (g day^{-1}) from leaf litter of each tree species, with 99% confidence intervals placed around means. Differences in letters above two means indicates statistical significance (p < 0.001).

until the point when the stream stopped flowing and began to dry (Fig. 5).

While the plastic litter bags utilized in this study probably provided good estimates of leaf mass loss brought about by microbial decomposition and physical fragmentation, they may not be adequate tools for estimating true breakdown rates in this system. Preliminary analysis of macroinvertebrate samples from litter bags reveals a paucity of insects that feed directly on CPOM. Insects were present in leaf litter bags, but the majority of them were not shredders. However, large

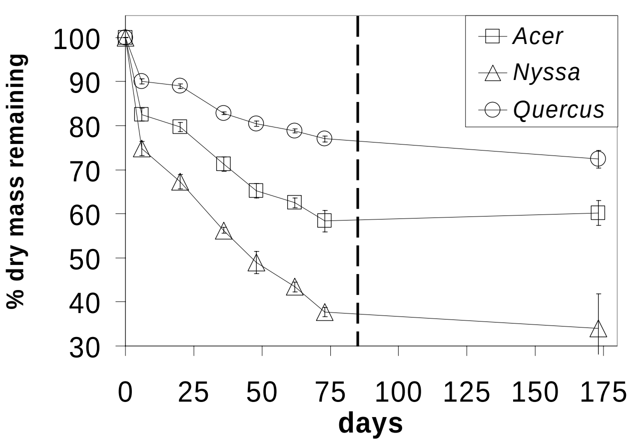

Figure 5. Percent dry mass of leaf litter remaining through time. Vertical dashed line indicates point at which stream was drying and leaves were exposed.

crayfish seemed to be abundant in the system, as they were encountered clinging to the outside of litter bags, and in woody debris dams and leaf packs within the stream. Their exclusion from leaf litter bags may have resulted in an underestimation of rates of mass loss, as crayfish have been demonstrated to be important consumers of leaf litter in other studies (Creed and Reed 2004, Nisikawa 2000, Schofield et al 2001). Leaves of *A. rubrum* and *Q. nigra* broke down quite slowly, loosing roughly 25 and 40 percent (respectively) of their total mass over the course of the incubation period. However, intact leaves of *A. rubrum* could not be found in the dry stream channel, and only a few scattered *Q. nigra* leaves remained.

CONCLUSIONS AND RECOMMENDATIONS

The findings of this preliminary study have provided useful information in the development of our budget of CPOM and dissolved oxygen dynamics. Effects of tree species on microbial respiration and mass loss were significant enough to warrant separation of riparian inputs according to species. The effects of temperature and time of incubation also suggest that these should be incorporated into our budget when estimating the magnitude of oxygen demand generated by CPOM. Thorough analysis of preserved microbial samples should provide useful information to further explain differences in respiration among levels of the various treatment factors in this study.

In the further development of our budget, we will examine effects of tree species not yet accounted for such as *Taxodium distichum* (Bald cypress), which is co-dominant with *Nyssa* sp. in higher-order reaches of the Little River. Further quantification of the role of crayfish in organic matter processing is also planned, and it is our hope that we will be able to produce a thorough explanation of how the biotic component of this system affects and is affected by dissolved oxygen and CPOM dynamics.

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