Effects of Nutrient Enrichment on Yellow Poplar and White Oak Leaf Decomposition and Fungal Activity in a Southeastern Stream

Monica Moss

Under the supervision of Dr. Suberkropp

We examined the effects of increasing nutrient concentration in the water of a soft water, Coastal Plain stream on decomposition rates of yellow poplar, and white oak leaf disks as well as on fungal growth and sporulation associated with this leaf material. The study was carried out in triplicate groups of flow-through channels placed in the stream. Treatments in each group of channels included enrichments with potassium phosphate, ammonium chloride, both nutrient, and no enrichment. During the study, ambient nutrient concentrations in the stream were: nitrate-N 20-35 ug/L; ammonium-N 5-60 ug/L, and phosphate-P 0-11 ug/L. Decomposition rate of yellow poplar leaf disks was greater than that of oak and was stimulated in treatments enriched by N+P as well as P. Oak decomposition rates varied little among treatments. Fungal growth rates calculated from increases in ergosterol concentrations were faster on yellow poplar than on oak. Ergosterol concentrations associated with both leaf types increased more rapidly in the treatments enriched by N+P and P suggesting that phosphorus was potentially limiting fungal activity in the stream.

Objective

The main objective was to examine experimentally whether dissolved inorganic P, N, or both nutrients in the water limited the activity of aquatic fungi colonizing leaves and, if so, whether the response of fungi differed on leaves of different quality. Yellow poplar (*Liriodendron tulipifera*) was chosen as the fast decomposing leaf type and white oak (*Quercus alba*) as the slow decomposing leaf type. Yellow poplar leaves are expected to decompose more quickly than white oak leaves, which have less available labile carbon and are thus harder to decompose. We carried out nutrient enrichments by adding nutrients to experimental flow-through channels positioned in Payne Creek in July 2000. Payne Creek is located in the Oakmullgee Wildlife Management Area of the Talledaga National Forest, AL, upstream of the University of Alabama's Talledega Wetland Ecosystem study site. Effects of nutrient enrichment will be determined on rates of leaf decomposition, ergosterol concentrations, and rates of fungal sporulation associated with the two types of leaves.

Materials and Methods

The experimental channels used were PVC pipes (7.5 cm id x 92 cm long) fastened together in groups of four channels (one for each treatment, see next paragraph,). Three replicate groups of channels were used and were placed 50-100 m apart in Payne Creek. Each channel has 4 semicircular baffles positioned at right angles in the upstream end to mix entering stream water with added nutrients.

The four treatments used included: a control, in which no nutrients were added; b) +N, in which NH_4CL was added; c) +P, in which equimolar quantities of KH_2PO_4 and K_2HPO_4 were added; and d) +N+P, in which both NH_4Cl and K_xH_xPO4 were added. Nutrients were added as concentrated solutions that were siphoned through ca 3m of small-bore (0.56 mm id) Teflon tubing.

At each sampling time, water from the experimental channels was filtered (GF/F) in the field and analyzed for phosphate, ammonium, and nitrate. Concentration of SRP was determined with ascorbic acid method (AHPA 1989), ammonium was determined with a fluorimetric method (Holmes et al. 1999), and nitrate was determined with an ion chromatograph. Concentrations of nutrients in the channel are given in Table 1.

The experimental sampling unit was a group of 5 leaf disks (12.5 mm diam), each pierced through their center by a stainless steel insect pin and held apart by glass bead spacers (ca 2 mm). Leaf disks were cut from autumn-shed yellow poplar and white oak leaves, leached 4 days in distilled water at 25°C with daily changes of distilled water. Pins containing leaf disks were pushed into corks (size 00) which were placed in hole drilled in Plexiglas plates. Plexiglas plates, each containing 39 pins, were placed in the downstream portion of the channels.

The response variables measured are: a) ash-free dry mass (AFDM) remaining - leaf disks (5) from a pin were dried at 100°C and then ashed at 500°C. Ten sets of leaf disks which were not placed in the stream were used to convert air dried mass to AFDM; b); fungal sporulation rates were determined by placing leaf disks (5) from a pin in aeration chambers and aerating for 20-30 hrs at 15°C in 40 ml stream water (Fig. 1). Aliquots of water were filtered, stained, and examined microscopically to determine the number and species composition of spores produced (Suberkropp 1991); and c) ergosterol concentrations - ergosterol was extracted from leaf disks (5) stored in alkaline methanol by refluxing for 30 min, partitioning into pentane, drying and redissolving in methanol (Suberkropp & Weyers 1996). Ergosterol was analyzed with an HPLC. At each sampling time, pins containing disks were removed from triplicate channel sets for each measurement except when they were unavailable due to loss in the stream flow or shredder (mostly crayfish) consumption.

Differences in decomposition rates (k) determined from linear regressions of ln transformed data were detected by analysis of covariance (ANCOVA) followed by Tukeys comparison. Ergosterol and sporulation were examined by two-way repeated measures ANOVA. If a significant treatment effect was found, one-way ANOVA was done for each date. Values of $p< 0.05$ were considered significant.

Results and Discussion

The nutrient additions enriched the stream water in the experimental channels – see Table 1. Payne Creek has low concentrations of SRP, but moderate concentrations of ammonium-N and nitrate-N, suggesting that organisms in this stream would potentially be limited by P.

During the experiment, the stream experienced low water flow common during the summer months in this area. Because of this factor, fewer nutrients than expected were taken up by stream organisms between the channel sets, so nutrients from the upstream setup partially contaminated the downstream channels. This increased variability in the nutrient enrichment, making the downstream channels more enriched than the upstream. Also during the experiment, small Mad Tom fish inhabited the channels, yet did not appear to upset the leaf disks. Unfortunately, crayfish who also inhabited the channels did do some damage to leaf disks, mostly white oak later in the experiment. This damage, however, is not considered significant because available uneaten leaves were used or adjustments made for this loss. Both yellow poplar (Fig. 1) and white oak leaf (Fig. 2) decomposition appeared to be potentially limited by P in this stream. Carbon quality did not affect which nutrient was limiting but affected the rate of decomposition. Statistical analysis of decomposition rates showed a significant difference from the control and N addition for P and N+P addition for yellow poplar (Table 2). Decomposition rates for white oak P, and N+P addition were significantly different from the control, while N addition was not significantly different from control or P and N+P addition (Table 2). Sporulation rates associated with yellow poplar were higher for N+P addition than for other treatments on day 5 (Fig. 3). Statistical analysis of sporulation rates for yellow poplar showed a significant difference for P addition. Sporulation rates associated with white oak were higher for N+P addition than for other treatments on day 5 (Fig. 4). Statistical analysis of sporulation rates for white oak showed a significant difference for P addition, as well as a significant difference for N+P addition at day 5. Ergosterol concentrations of yellow poplar leaf samples suggest a greater increase in fungal biomass at day 5 for N+P addition (Fig. 5). Statistical analysis of ergosterol concentrations for yellow poplar leaf samples show a significant difference for P addition. Ergosterol concentrations of white oak leaf samples do not appear to show a greater increase in fungal biomass for nutrient addition (Fig. 6). Statistical analysis of ergosterol concentrations for white oak shows no significant treatment effect. Overall, the data suggests that fungal activity in this stream may be limited by P concentration.

Nutrient	Treatment			
	Control	+P	+N	+N+P
Phosphate-P	8(0-11)	57(32-87)	7(3-11)	63(35-98)
Ammonium-N	30(5-57)	27(5-50)	143(71-252)	158(76-246)
Nitrate-N	26(22-34)			

Table 1. Concentrations of nutrients and temperature during nutrient enrichment at ~25˚C
Values given are means(□g/L) with ranges of concentrations given in parentheses.

Treatment	k -poplar	k –oak
Control	0.0197[a]	0.0209 [a]
Phosphorus	0.0470 [b]	0.0392 [b]
Nitrogen	0.0253 [a]	0.0279 [ab]
Nitrogen and Phosphorus	0.0576 [b]	0.0324 [b]

Table 2. Decomposition rates (k) of yellow poplar and white oak leaves. Different letters indicate significant differences, ANCOVA.

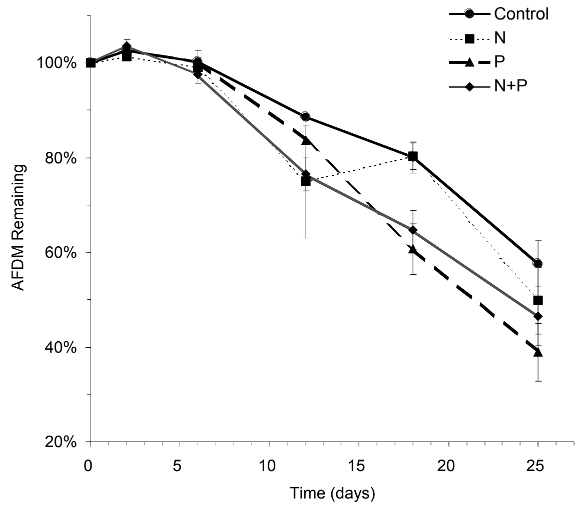

Figure 1. Effect of nutrient addition on decomposition of yellow poplar leaves.

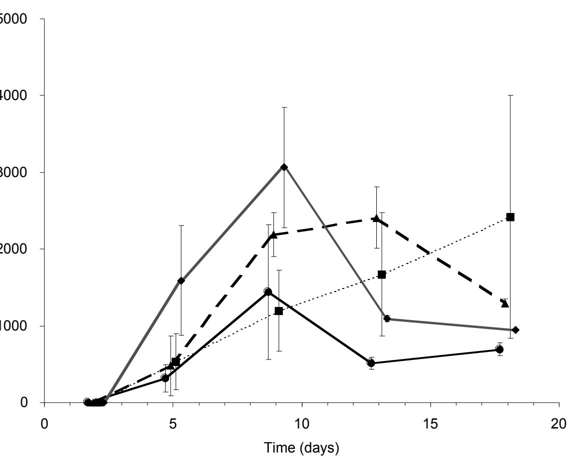

Figure 2. Sporulation rates associated with yellow poplar leaf samples

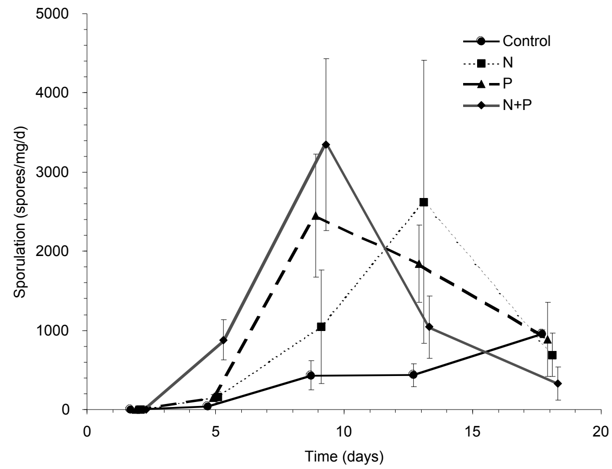

Figure 3. Sporulation rates associated with white oak leaf samples.

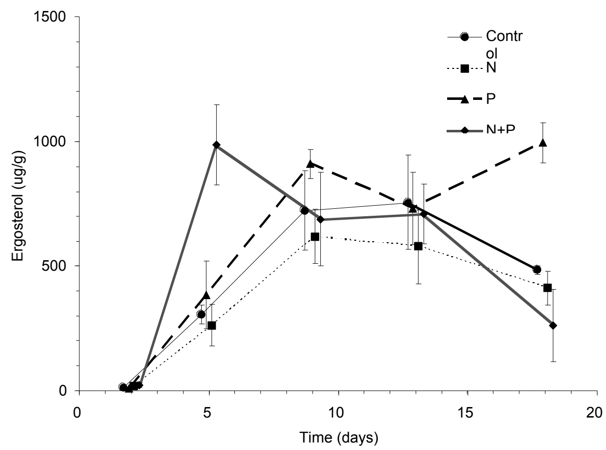

Figure 4. Ergosterol concentration of yellow poplar leaf samples.

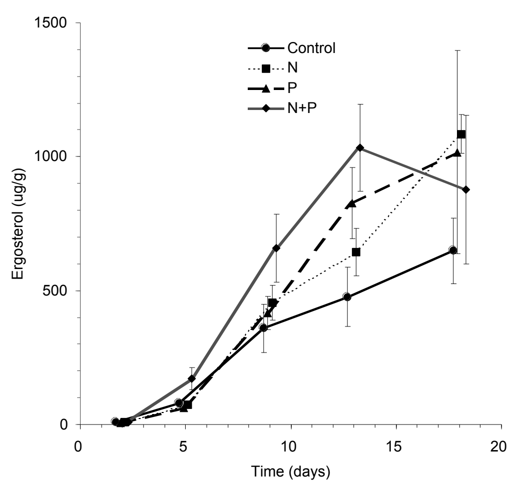

Figure 5. Ergosterol concentration of white oak leaf samples.

References

APHA. 1989. Standard methods for the examinations of water and waste water, 17th ed., American Public Health Association, Washington, D. C., USA.

Holmes, R. M., A. Aminot, R. Kerouel, B. A. Hooker & B. J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 56:1801-1808.

Suberkropp, K. 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. *Mycological Research* 95:843-850.

Suberkropp, K. & H. Weyers. 1996. Application of fungal and bacterial production methodologies to decomposing leaves in streams. *Applied and Environmental Microbiology* 62:1610- 1615.

Monica Moss graduated with a degree in biology with a focus in ecology in the spring of 2002. She was president of the Alabama Environmental Council. This project was funded by a summer internship from the Howard Hughes Medical Institute Undergraduate Biological Sciences Education Program Grant awarded to The University of Alabama in coordination with Stillman College.