# LINKING HETEROTROPHIC METABOLISM AND NUTRIENT UPTAKE IN HEADWATER STREAMS

Travis Gray

Thesis submitted to the faculty of Virginia Polytechnic Institute and State University In partial fulfillment of the requirements for the degree of

> Master of Science in Biological Sciences

H.M. Valett, Chair E.F. Benfield W.L. Daniels

25 April 2007 Blacksburg, Virginia, USA

Keywords: metabolism, nutrient demand, nitrate uptake, nutrient spiraling, allochthonous organic matter, ecosystem, litter breakdown

## LINKING HETEROTROPHIC METABOLISM AND NUTRIENT UPTAKE IN HEADWATER STREAMS

#### **Travis Gray**

#### Abstract

Autotrophs and heterotrophs differ in their demand, acquisition and use of materials, but fundamentally nutrient demand is inherently linked to metabolism based on the stoichiometry of biochemical reactions. The differences between these two groups of organisms confound straightforward regression approaches to quantifying the relationship between nutrient demand and metabolism at an ecosystem level. We address how nutrient demand in headwater streams changes with shifts in organic matter supply and associated microbial activity by investigating these relationships in the predominantly heterotrophic conditions of a southern Appalachian stream. We measured litter input, organic matter standing crops, litter respiration rates and nitrate demand several times during the course of decomposition. There was a strong relationship between leaf standing crop and nitrate uptake efficiency across dates with maximal efficiency occurring when litter standing crops were highest. There was also an increase in nitrogen (N) uptake rate relative to respiration rates as breakdown progressed, which appears to be due to a shift in nutrient supply from the substrate to the water column associated with the depletion of labile, high quality organic matter in the substrate. It is our contention that streams establish a gradient of resource supply from particulate to dissolved sources that coincides with the movement of materials from terrestrial to marine systems.

# **Grant Information**

This research was supported by grants SRS 04-CA-11330139-164 and SRS 03-CA-11330139-202 from the USDA Forest Service, grant DEB0425624 from the National Science Foundation, and two Graduate Research Development Plan grants from the Virginia Tech Graduate Student Association.

# Acknowledgments

I would like to thank all members of the Stream Team of Virginia Tech who have assisted with various aspects of this project. This work is certainly in part a reflection of their instrumental help with field work and lab analyses as well as the lively debates in which we have engaged for the last three years.

# **Table of Contents**

Abstract	ii
Grant Information	iii
Acknowledgments	iv
Table of Contents	v
List of Figures	vi
List of Tables	vii
Introduction	1
Methods	3
Study sites and dates	3
Organic matter	3
Solute releases	4
Uptake metrics	5
<i>Microbial respiration</i>	5
Modeling the link between metabolism and N demand	6
Data Analysis	8
Results	8
Reach-scale patterns	8
Litterfall	9
Leaf biofilm respiration	9
Modeling microbial function and efficiency	10
Discussion	11
Ecosystem N demand	11
Linking heterotrophy and N demand	12
Literature Cited	17

# **List of Figures**

Figure 1. Temporal patterns in organic matter standing crops and chemical parameters. OM
data are means (± SE) for sampling dates. Different letters denote statistically significant
differences across dates 21
Figure 2. Relationship of $v_f$ and U with OM standing crops. Left panels (A and C) are versus
CBOM standing crop and right panels (B and D) are versus Total OM (FBOM + CBOM) 22
Figure 3. Leaf disk respiration rates over the course of litterfall and decomposition. A) Rates
measured at laboratory temperature (~20°C). B) Rates after $Q_{10}$ transformation with $Q_{10}=2.5$ .
Symbols (means $\pm 1$ SE) are (•) Birch, ( $\circ$ ) Tulip Poplar, ( $\mathbf{\nabla}$ ) Beech, ( $\triangle$ ) Oak, ( $\blacksquare$ )
Rhododendron
Figure 4. Measured (•) and model predicted () CBOM standing crops. Observed values are
black dots (means $\pm 1$ SE). Dashed line is modeled total stream CBOM standing crop24
Figure 5. Comparison of N demand predicted by the model and observed whole-stream
demand. Arrows indicate progress through time. Dotted line indicates the 1:1 line
<b>Figure 6</b> Calculated values of the microhial production coefficient (PR) respiratory quotient

# List of Tables

Table 1.	Physico-chemical variables on each sampling date	27
Table 2.	Litterfall and breakdown parameters used in modeling standing crops	28

### Introduction

Organismal nutrient demand is necessarily linked to metabolism by the stoichiometry of biochemical reactions. Universal metabolic processes (e.g. respiration) warrant similar enzymatic composition and hence analogous nutrient demand across multiple scales of biological organization. This results in broad patterns in the relationship between resource supply and metabolism both at the organismal (Hedin and others 1998) and ecosystem (Redfield 1958, Elser and others 1996) levels.

Several recent studies have attempted to link nutrient demand to metabolic measures in stream ecosystems with some success (Hall and Tank 2003; Webster and others 2003; Fellows and others 2006). All three previous studies relied heavily on whole-system metabolic measures across multiple sites and across biomes. Linking metabolism and nutrient demand may be complicated by this approach. Whole-system metabolic measures reflect the combined influence of both autotrophs and heterotrophs. These two groups perform fundamentally different functions (i.e. anabolic versus catabolic processes) with equally distinct biochemical reactions. Hence these groups should have dissimilar nutrient demand reflecting their metabolic character (Sterner and Elser 2002). Further, Webster and others (2003) noted that it may be difficult to find relationships between functional parameters across ecosystems due to the functional diversity of microbial communities (e.g. assimilative vs. non-assimilative uptake). Accordingly, simple linear relationships between ecosystem-level measures of metabolism and nutrient demand across systems with varying amounts of autotrophic and heterotrophic activity may not exist. An alternative approach focuses on autotrophic and heterotrophic activity separately and at the sub-reach scale. Since reach-level measurements of respiration (and hence GPP since it is dependent on respiration estimates using the two-station approach) may not be accurate estimates (Webster 2007), measurements of metabolism should also be made at a sub-reach scale.

Other factors may obscure links between metabolism and nutrient uptake when addressed across multiple sites. For example, differences in the quality of organic matter available to heterotrophs may confound results and promote conclusions that conflict with prevailing knowledge. Hall and Tank (2003) were able to correlate ecosystem respiration to ammonium-nitrogen (NH<sub>4</sub>-N) demand across 10 streams in the Grand Teton National Park, Wyoming, but then found no relationship between ecosystem respiration and nitrate-N (NO<sub>3</sub>-N) demand. They suggested that heterotrophic microbes in their study streams may not be capable of NO<sub>3</sub>-N

uptake. The fact that heterotrophic NO<sub>3</sub>-N demand has been measured not only in streams (Elwood, Mulholland, and Newbold 1988; Tank and others 2000; Fellows and others 2006) but in other ecosystems (Kirchman and Wheeler 1998; Stark and Hart 1997) indicates that heterotrophs retain some flexibility in their methods of nutrient acquisition, making it unlikely that they would entirely lack the capability to assimilate NO3-N at any single site, especially when N is available in very small supply (as was true for these Wyoming streams). Instead patterns observed across sites may be complicated by changing terrestrial-aquatic linkages (England and Rosemond 2004) or other compounding factors. Thus focusing on a single site simplifies these complex relationships allowing increased mechanistic understanding with the potential to then scale across sites and systems.

We employed this approach to understand the linkage between metabolism and nutrient uptake. We studied a single southern Appalachian stream through time, using N spiraling methods to quantify nutrient uptake and respiration measurements standardized by substrate mass to quantify the metabolic activity of heterotrophs. Appalachian streams provide an excellent system to study the relationship between heterotrophic metabolism and nutrient uptake due to minimal autotrophic activity (Webster and others 2003). These streams have especially close structural and functional connections to their highly vegetated riparian zones. Canopy vegetation and a thick understory of evergreen rhododendron combine to minimize insolation (Clinton and Vose 1996), keeping in-stream autotrophic production low throughout the year. Further, the forest canopy delivers an annual autumnal pulse of organic matter that fuels detritalbased food webs (Wallace and others 1997). While heterotrophs rely on these annual subsidies as organic carbon energy sources, the high carbon to nitrogen (C:N) ratios of the leaves (~ 50; Cross and others 2005) compared to microbes (~5-15; Cross and others 2005) make them comparatively poor sources of N. Instead N is sequestered from inorganic sources in the water column, inherently linking nutrient uptake to organic matter dynamics in these streams (Mulholland and others 1985, Webster and others 2001b).

In this study we took advantage of the annual flux of resources into Appalachian streams to address how the supply and decomposition of allochthonous organic matter influences nutrient uptake during autumn. We especially focused on the role of heterotrophs as an important vector for NO<sub>3</sub>-N removal from the water column and to resolve the link between whole-stream nutrient demand and microbial function. Metabolic oxygen demand was measured on decaying leaf

material, scaled by leaf standing crop to obtain reach estimates, and related to reach-level nutrient uptake to assess relationships between metabolism and ecosystem N cycling. We predicted that if heterotrophic microbes were important determinants of stream N dynamics maximal nitrogen demand would be associated with maximum reach-level heterotrophic activity but not necessarily associated with peak organic matter (OM) standing crops. Further, changes in N demand should be dependent on changes in heterotrophic respiration rate.

### Methods

#### Study sites and dates

Research took place in a headwater stream within the Rays Branch watershed in the southern Appalachian Mountains, western North Carolina. Soils in this area are generally developed from biotite gneiss with locally abundant quartz and aluminum silicates. Streams in the area tend to be phosphorus (P) limited year-round (Gray, *unpublished data*). Native vegetation consists primarily of oak-hickory forest with Eastern Hemlock and White Pine. Rhododendron (*Rhododendron maximum*) was sparsely present at the stream margins along the study reach. A 100-m study reach was established in the stream at the beginning of autumn. Sampling began before litterfall (October 1, 2005) and continued through winter (February 18, 2006) as in-stream processing of allochthonous inputs occurred. In general, we quantified OM inputs and standing crops, used solute releases to quantify N uptake, and measured microbial respiration on leaf litter in laboratory microcosms. Finally, we developed a simulation model to link metabolic and biogeochemical processes and assess potential controls over this association.

#### Organic matter

Standing crops of fine benthic organic matter (FBOM, particles <1 mm) and coarse benthic organic matter (CBOM, particles >1 mm) were quantified following solute injections. At least 5 samples of each were collected using a stratified random sampling design along the stream reach. CBOM was collected by sealing a metal cylinder around the stream bottom and removing all visible particles in the enclosed area. In the lab woody material was removed from the sample and remaining OM was weighed, ground, sub-sampled, and combusted to determine standing crop as ash free dry mass (AFDM). We then scaled standing crop by area to obtain g AFDM m<sup>-2</sup>. FBOM was collected with the same approach after removing CPOM. The upper

5cm of sediment was agitated and a sample of the suspension collected for analysis. Depth of water in the cylinder was measured to determine total volume of the sample. The FBOM sample was sieved to remove large particles, sub-sampled, and filtered (Whatman GF/F, 0.45µm pore size). Filters were combusted to determine OM as g AFDM. Concentrations of AFDM in the sub-sample were converted to g AFDM m<sup>-2</sup> using depth of water in the cylinder.

Litterfall (g AFDM m<sup>-2</sup> d<sup>-1</sup>) inputs were measured along the study reach using litterfall collection buckets suspended over the stream (10 buckets along 100 m). The bottoms of the buckets were lined with wire mesh to suspend the sample and minimize leaching. Samples were retrieved periodically (7 times over the study). Litterfall was separated by species, dried, and weighed to determine species-specific areal input rates to the stream. A total of 11 species were identified and their litterfall rates independently tracked. All litter from other species or deemed unidentifiable due to condition were classified as miscellaneous.

#### Solute releases

Reach-scale measures of nutrient uptake were quantified using solute release experiments (Stream Solute Workshop, 1990). Stream discharge at the time of sampling was measured using a chloride slug prior to the injection (Kilpatrick and Cobb 1985). Prior to release, triplicate background water samples were collected at each of 7 transects distributed along the 100-m reach. Sodium nitrate (NaNO<sub>3</sub>) was released along with a conservative tracer (chloride as NaCl) at a constant rate until the reach was well-mixed throughout the reach (i.e., plateau conditions) as evidenced by a stable specific conductance at the downstream-most transect. Target enrichment was 75  $\mu$ g L<sup>-1</sup> NO<sub>3</sub>-N (100% enrichment of pre-autumnal background concentrations). Longitudinal decline in the concentrations of both solutes was measured using triplicate plateau samples taken from each transect. Samples were filtered in the field using glass fiber filters (Whatman GF/F, 0.45 $\mu$ m pore size) and kept on ice during transport to the laboratory for analysis. Measurements of stream width (*w*, m) and depth (*z*, cm) were made randomly across 10 transects within the reach.

In the laboratory, NO<sub>3</sub>-N and chloride (Cl<sup>-</sup>) were measured by ion chromatography on a Dionex DX500 (Dionex, Sunnyvale, California, USA). On several dates NO<sub>3</sub>-N was below 25  $\mu$ g L<sup>-1</sup>. These samples were analyzed colorimetrically following cadmium reduction (Wood and others 1967, APHA1998) on a Technicon Autoanalyzer (Technicon, Emeryville, California,

USA). Ammonium-N was determined using a modified phenol-hypochlorite method (Solorzano 1969; USEPA 1997). Dissolved organic carbon (DOC) was determined by oxidative reaction with sodium persulfate using a total carbon analyzer (Model 1010, OI Analytical, College Station, Texas, USA; Menzel and Vacarro 1964; APHA 1998). Concentrations below detection were recorded as half the detection limit.

# Uptake metrics

Plateau NO<sub>3</sub>-N was corrected for background concentrations and dilution as measured by the decline in the concentration of the conservative tracer (Stream Solute Workshop 1990). Natural-log transformed corrected concentrations were regressed against distance downstream and the slope of the regression ( $k_L$ , m<sup>-1</sup>) was used to determine uptake length ( $S_w$ , m) by Equation 1. Uptake velocity ( $v_f$ , mm min<sup>-1</sup>) was then calculated by Equation 2 and areal uptake rates (U, g m<sup>-2</sup> d<sup>-1</sup>) were derived from  $v_f$  and background NO<sub>3</sub>-N ( $\mu$ g L<sup>-1</sup>) by Equation 3 where numeric values are conversion factors for unit compliance.

$$S_{w} = \frac{1}{k_{L}} \qquad (1)$$

$$v_{f} = \frac{Q * k_{L} * 60}{w} \qquad (2)$$

$$U = \frac{v_{f} * [NO_{3} - N]_{bkg} * 1440}{1000^{2}} \qquad (3)$$

### Microbial respiration

Respiration rates were measured to quantify leaf biofilm metabolic activity for the 5 dominant leaf species in the stream including: Beech (*Fagus grandifolia*), Birch (*Betula lenta*), Oak (*Quercus* spp.), Tulip Poplar (*Liriodendron tulipifera*) and Rhododendron. Before initiating nutrient releases, stream water and leaves were collected from the study reach for use in the laboratory. Leaves were collected using a stratified random approach throughout the reach. Leaves were kept in containers full of stream water and all samples were placed on ice for transportation to the laboratory. In the lab, leaves were separated by species and discs of uniform size (1.6 cm diameter) were cut from the dominant species. Initial dissolved oxygen (DO) concentration of water used for respiration assays was determined by titrating stream water

using a modified Winkler method (Hauer and Hill, 1996). Leaf discs were placed in 30mL flasks filled with stream water, capped without headspace, and allowed to incubate in darkness for at least 8 hours at room temperature. Control flasks of stream water without leaves were incubated simultaneously to correct for seston respiration. After incubation the flasks were titrated to determine DO concentration. Oxygen consumption was calculated as the change in DO concentration after correcting for controls. Leaf discs were dried, weighed, and ashed to determine AFDM. Respiratory rate was expressed as mg  $O_2$  g AFDM<sup>-1</sup> d<sup>-1</sup>. A Q<sub>10</sub> transformation was used to relate rates at room temperature to rates at stream temperature by Equation 4 where R is the respiration rate in the specified environment, T is the temperature (°C), and Q<sub>10</sub> is a constant relating temperature-specific rates. We applied a Q<sub>10</sub> of 2.5 based on previous work examining the effects of temperature on respiration in microbial communities (Liu and others 2006; Hancke and Glud 2004).

$$\mathbf{R}_{\text{stream}} = \mathbf{R}_{\text{room}} \mathbf{Q}_{10}^{(T_{\text{stream}} - T_{\text{room}})/10}$$
(4)

On some dates we were unable to find any adequate samples of the target leaf species. In these cases we used an average of the respiration rates for that species on the sampling dates immediately before and after the missing date. For other leaves in the stream (i.e. Miscellaneous) a simple average of the respiration rates on the five target species was applied.

#### Modeling the link between metabolism and N demand

Scaling measured leaf respiration rates to whole-stream CBOM metabolism required addressing species-specific contributions to in-stream leaf biomass on a given sampling date. This was not possible empirically because the relatively quick fragmentation of some leaf species (e.g., Maple and Birch; Webster and Benfield 1986) renders them unidentifiable long before they cease to represent a significant portion of the detrital standing crop. Instead we applied a model developed to assess standing crops for mixed species (Webster and others 2001a) and adapted it to predict species standing crops through time. Changes in leaf standing crop for each species were estimated by Equation 5 where *dt* is the change in time,  $M_j$  is the standing crop of the j<sup>th</sup> species, *t* is time, I(j,t) is the input of the j<sup>th</sup> species to the stream at time t,  $k_j$  is the decay rate of the j<sup>th</sup> species, and  $T_t$  is the temperature at time t.

$$\frac{dM_j}{dt} = I(j,t) - k_j T_t M_j dt$$
(5)

Input was calculated as 118% of the measured species-specific litterfall rate to account for lateral blow-in (Webster and others 1990). Species-specific decay rates  $(day^{-1})$  were estimated based on published measurements from nearby Coweeta Hydrologic Laboratory streams (Webster and Benfield 1986; Webster and others 1999). Literature values from reference watersheds were multiplied by 1.5 based on faster breakdown rates observed in other secondary successional watersheds in the region (Benfield and others 2001). To include thermal influences on decomposition, rates were transformed to proportional loss per degree day ( $k_j$ ; degree day<sup>-1</sup>) by dividing by the average daily temperature of the stream (Webster and others 2001a). In-stream litter transport was considered negligible (Webster and others 1999; Webster and others 2001a).

CBOM standing crops were sampled in spring as part of a larger study. We used these values to parameterize the initial total standing crop such that modeled standing crops corresponded to observed standing crops on that date. Initial proportions of each species within the initial total standing crop were parameterized by running the model repeatedly using the resulting proportion of each leaf species after one year as the initial proportion in the next run until equilibrium was achieved.

Modeled standing crops were used to determine areal respiration rate ( $R_{area}$ ) by Equation 6 where *S* is the total number of species and  $R_{jt}$  is the respiration rate of the j<sup>th</sup> species at time t as determined by incubations described above. N demand was then calculated using areal respiration rates by Equation 7 where 12 and 32 are molar conversions, *RQ* is the respiratory quotient relating moles of CO<sub>2</sub> produced to moles of O<sub>2</sub> consumed (0.85; Bott, 1996), *PR* is the microbial production ratio determining biomass production as a fraction of respiration (0.28; Cole and Pace 1995), *CN<sub>microbes</sub>* is the molar C:N of the microbial assemblage, and  $U_{calc}$  is the predicted areal uptake flux of N (g m<sup>-2</sup> d<sup>-1</sup>).  $U_{calc}$  was also used to calculate a predicted  $v_f$  using background NO<sub>3</sub>-N (Equation 3) to allow comparisons of both N uptake efficiency ( $v_f$ , Valett and others 2002) and areal flux to observed values.

$$R_{area} = \sum_{j=1}^{S} R_{jt} M_{j} \quad (6)$$
$$U_{calc} = \frac{R_{area} * \frac{12}{32} * RQ * PR}{CN_{microbes}} \quad (7)$$

## Data Analysis

Differences in CBOM and FBOM across dates were assessed with a one-way ANOVA using sampling date as the main effect with Tukey's HSD post-hoc tests to determine differences among dates when significant. Comparison of leaf respiration rates among dates and species required an unbalanced design due to the fact that on some dates no intact specimens were found (e.g. Beech fell late in the season). Accordingly we analyzed leaf respiration rates using an unbalanced ANOVA design and used Type III SS to test the effect of species and date. Relationships between physico-chemical variables across dates were evaluated using linear regression analyses. Model fit was assessed by computing 95% CBOM confidence intervals for each date sampling was performed and determining how many model CBOM predictions fell within those intervals. All tests were conducted using SAS 9.1 (SAS Institute, Cary, NC) and were considered significant at  $\alpha = 0.05$ .

# Results

#### *Reach-scale patterns*

On all sampling dates discharge was less than 15 L s<sup>-1</sup> and varied less than an order of magnitude with magnitude decreasing through autumn and increasing in January (Table 1). At the same time DOC decreased continually through the season from 0.58 to 0.29 mg L<sup>-1</sup> with the exception of one sampling date when concentrations were more variable (Table 1). NO<sub>3</sub>-N was closely correlated to stream discharge (r = 0.87, p = 0.011), decreasing from 37  $\mu$ g L<sup>-1</sup> at the beginning of the season to 1  $\mu$ g L<sup>-1</sup> on November 21<sup>st</sup>, then subsequently increasing on the last two sampling dates to 57  $\mu$ g L<sup>-1</sup> (Figure 1).

CBOM standing crops were highly variable during autumn, ranging more than an order of magnitude from 15 to 254 g AFDM m<sup>-2</sup> (p = 0.0009; Figure 1). Lowest standing crops occurred at the beginning of autumn and highest standing crops occurred in November before decreasing later in the season. While highest FBOM standing crops were coincident with highest CBOM standing crops, FBOM standing crops ranged only 117 from 41 to 158 g AFDM m<sup>-2</sup> (p = 0.284; Figure 1) with highest standing crops also occurring in November. In general, minimal stream NO<sub>3</sub>-N was observed during times of maximal CBOM standing crop (Figure 1), however NO<sub>3</sub>-N was not related to mean CBOM standing crops (r = 0.43; p = 0.339) due to especially high NO<sub>3</sub>-N and high CBOM standing stocks occurring on the last date. FBOM and NO<sub>3</sub>-N did not appear to be related due to increased FBOM standing crops during mid-season (Figure 1).

N demand, as measured by *U*, increased from 0 to 0.029 g N m<sup>-2</sup> d<sup>-1</sup> over the course of the study (Figure 1); however it did not begin to increase substantially until mid-autumn when CBOM standing crops began to decline and NO<sub>3</sub>-N increased. N uptake efficiency ( $v_f$ ) also tended to increase over the course of the study from 0 to 0.67 mm min<sup>-1</sup>; however highest  $v_f$  values were observed with highest CBOM standing crops and  $v_f$  then decreased until the end of the study as CBOM standing crops declined. There was a strong positive correlation between  $v_f$  and both standing crop of CBOM (r = 0.96; p = 0.0004; Figure 2A) and total OM standing crop (r = 0.89; p = 0.007; Figure 2B). However due to the influence of NO<sub>3</sub>-N in its calculation, *U* was not significantly related to any measures of OM standing crop (Figure 2 C,D).

### Litterfall

Tulip Poplar was the dominant species in litterfall accounting for 25% of total inputs (Table 2). The five species on which we measured respiration rates (Beech, Birch, Oak, Tulip Poplar, and Rhododendron) accounted for 64% of total inputs to the stream. Leaves from species that either we did not track or were unidentifiable (i.e., Misc leaves) made up less than 8% of total measured litter inputs. Some species tended to fall at relatively constant rates throughout sampling (e.g., Tulip Poplar) while others were most commonly found on later sampling dates (e.g. Chestnut and Beech). Litter that tended to contribute more to inputs on later dates was more refractory (see Webster and Benfield 1986). During the 3 weeks ending November 19<sup>th</sup>, Chestnut, Beech, Red Maple, Oak, and White Pine each contributed over 55% of their total seasonal inputs. As a result, most litterfall occurred before the November 19<sup>th</sup> sampling and litter was absent from collection devices after December 3<sup>rd</sup>.

#### Leaf biofilm respiration

On any single sampling date we were able to quantify the respiratory activity of 23-61% of the litter standing crop in the stream (based on model estimates of species standing crops). Leaf disk respiration rates varied 16–fold across all species and dates  $(2.3 - 37.1 \text{ mg O}_2 \text{ g} \text{ AFDM}^{-1} \text{ d}^{-1}$ ; Figure 3A). Rates tended to decrease over the course of the study (p<0.0001) but there was a high degree of variability between species both on (p<0.0001) and among specific

dates (p<0.0001) that confounded post-hoc multiple comparison techniques for comparing rates across species or dates. The highest and most variable respiration rates were observed on November 19<sup>th</sup> after which lower rates coincided with the period of minimal litterfall (Table 2) and highest CBOM standing crops (Figure 2). By the final sampling date litterfall had ceased and respiration rates were much less variable both between and within species. After the Q<sub>10</sub> transformation was applied the same general patterns held but the range between minimum and maximum rates increased to 30–fold (0.6 - 18.1 mg O<sub>2</sub> g AFDM<sup>-1</sup> d<sup>-1</sup>; Figure 3B). More labile species (Webster and Benfield 1986) tended to have higher respiration rates than more refractory species.

The period of maximum variability in respiration rates corresponded to the dates during which no N demand was measured. Although respiration rates tended to decrease throughout the study, overall N demand (U) increased monotonically. However uptake efficiency tended to be inversely related to a simple average of  $Q_{10}$  transformed respiration rates (r=0.90, p=0.0154).

# Modeling microbial function and efficiency

Parameterization of the model resulted in an initial standing crop of 63 g AFDM m<sup>-2</sup> on January 1<sup>st</sup>, 2005. Initial standing crop was chosen so that the total standing crop in the modeled stream would be equal to the observed standing crop on the spring sampling date (45 g AFDM m<sup>-2</sup>). It was not possible to validate model predictions for individual species against field data because of the difficulty in identifying fragmented leaf material to species. However we were able to compare the predicted total standing crops to the measured values (Figure 4). Model predictions for all dates were within 95% confidence limits calculated from the CBOM samples on that date. When CBOM standing crops were high the model was very accurate, predicting inside the bounds of the sample means  $\pm 1$  standard error. For lower CBOM standing crops the model consistently over-estimated the sample mean by an average factor of 3 (excluding summer when more than half of CBOM samples were 0).

Across the study period, predicted U and  $v_f$  were within an order of magnitude of observed values with an average error of 3-fold (Figure 5). Estimates of both parameters were most accurate in January; however predicted values were generally different from empirical measures of N demand and the nature of estimate error changed over time. Lower than expected  $v_f$  values occurred early in the season but by February the model over-predicted  $v_f$  by . Since U

is a simple transformation by nutrient concentration it exhibited the same progression from lower to higher than expected values, however a plot of observed U versus predicted resulted in a linear relationship as opposed to the circular pattern of  $v_{f}$ .

# Discussion

#### Ecosystem N demand

We observed strong differences in intra-seasonal whole-stream N demand which we attribute to OM dynamics. The range of NO<sub>3</sub>-N  $v_f$  values observed were similar to those from other studies of NO<sub>3</sub>-N uptake in the southern Appalachian region (Earl, Valett and Webster 2006) and elsewhere (Hall and Tank 2003). Maximum *U* and  $v_f$  were observed when CBOM standing crops were greatest and NO<sub>3</sub>-N was lowest. Peak nutrient demand has been observed during autumn in other studies in the southern Appalachians for both N (Mulholland 2004) and phosphorus (Mulholland and others 1985; Webster and others 2001b; Mulholland 2004). In the past, high autumnal nutrient demand has been attributed to greater heterotrophic activity on CBOM (Elwood, Mulholland, and Newbold 1988; Tank and others 2000) and the relationship between  $v_f$  and CBOM standing crop in our study supports this general conclusion. Although FBOM standing crops should also contribute to heterotrophic activity, there was no relationship between FBOM and  $v_f$ . This is likely a result of the low quality of FBOM, the low metabolic activity levels associated with this compartment, and high turnover time due to transport relative to CBOM (Webster and others 1999; Cross and others 2005).

The strong relationship between CBOM standing crop and  $v_f$  in this study stands in contrast to inter-site studies that have found statistically weak or non-existent relationships between these two variables (Webster and others 2003). Our assertion that there may be important local drivers of nutrient demand that confound simple two-parameter relationships such as that of respiration and nutrient demand across systems seems to be supported by this evidence. The strong relationship between these two variables also suggests rapid responses of stream ecosystems to inputs of organic matter since we detected no time lag between standing crops and N demand. Sampling on a bi-weekly interval suggests that response of the microbial communities must occur on the order of several days or less (Gulis and Suberkropp 2002).

#### *Linking heterotrophy and N demand*

In this study we were able to identify significant demand for NO<sub>3</sub>-N associated with the breakdown of litterfall in mid to late autumn, a time of overwhelmingly heterotrophic activity in southern Appalachian streams. This supports our contention that heterotrophs are responsible for NO<sub>3</sub>-N demand in these systems but does not necessarily link N uptake and catabolic processes. A relationship between metabolism and nutrient demand has been documented in inter-biome (Webster and others 2003) and regional studies (Hall and Tank 2003), however these studies used ecosystem-level measures of metabolism which integrate autotrophic and heterotrophic metabolism. We specifically attempted to address measures of detrital pools and associated heterotrophic respiration to coincide with our instantaneous measures of reach-level N demand.

To establish patterns between metabolism and NO<sub>3</sub>-N demand, we scaled up measures of litter mass-specific heterotrophic activity to the whole-system level. The model of leaf decay we used to determine reach standing crops was tested more explicitly by Webster and others (2001a) in Hugh White Creek (HWC) at Coweeta Hydrologic Laboratory, North Carolina. Its close proximity and similar vegetation suggest underlying mechanisms of the model would are appropriate and applicable. On several dates we were able to predict CBOM standing crops within one standard error (Figure 4). Lower CBOM standing crops were consistently overestimated by the model and this may be expected given that our small sample sizes in this study (i.e., 5 in spring/summer, 10 in autumn) increases the chance of calculating a mean standing crop lower than the true value (Webster and others 2001a). Predicted proportions of each species comprising the total standing crop should also accurately reflect the composition of CBOM on each date since we directly measured litterfall inputs and the total standing crop predictions were generally accurate. Inter-specific differences in respiration rate on most dates corresponded to the expected relationship between leaf species based on breakdown rates and while respiration rates were not measured at stream temperature, temperature-corrected rates were within the range of rates observed in previous studies of leaf respiration conducted at Coweeta Hydrologic Laboratory (e.g. Gulis and Suberkropp 2003; Tank and others 1993) suggesting satisfactory transformation.

Excluding dates with no observed whole-stream uptake, predictions were within an order of magnitude of observed values (Figure 5) indicating a somewhat predictable relationship between NO<sub>3</sub>-N uptake and metabolism. Predicted values of U were higher than measured at the

beginning of autumn but by the last sampling date the model over-estimated uptake. Due to variation in NO<sub>3</sub>-N availability, this pattern was manifested as a circular rather than linear pattern in  $v_{f}$ . In general, our model captured the relationship between metabolism and uptake but evidently missed a temporal component of the association. By exploring what may have caused this pattern we should be able to infer more about how metabolism and N demand are linked in heterotrophic systems.

Modeling of both U and  $v_f$  shifted from over-prediction to under-prediction coincident with the cessation of litterfall, suggesting that inputs of fresh organic matter during litterfall may have biased the model toward over-prediction. However, Gulis and Suberkropp (2003) observed an approximate doubling of respiration rates on leaves that had decayed for 60-190 days compared to those that had only been in the stream for a short period (~15 days) in a reference portion of watershed 54 in Coweeta Hydrologic Laboratory. Applying a similar increase in respiration to older CBOM standing crops in our model would only serve to increase overprediction. We therefore contend that respiratory character of leaf cohorts was not central to the lack of fit between predicted and observed rates of N uptake.

Chapin (1980) measured the efficiency of nutrient use as the amount of organic matter produced per nutrient taken up. In this model we assumed that organic matter production was proportional to respiration and, in doing so, assumed constant nutrient use efficiency. If instead it changed during the course of decomposition, our predicted uptake rates would be incorrect. To examine this possibility we can calculate a respiratory nutrient use efficiency (NUE<sub>R</sub>) defined as units of respiration per unit of nutrient uptake. Then from equation 7:

$$NUE_{R} = \frac{R_{area}}{U_{obs}} = \frac{\frac{32}{12} * CN_{microbes}}{RQ * PR}$$
(8)

Thus changes in NUE<sub>R</sub> during the course of decomposition would be manifested as changes in model parameters estimated from the literature (i.e. RQ, PR, and  $CN_{microbes}$ ). NUE<sub>R</sub> was infinite on the first 2 dates due to no measured N uptake. It then decreased through the study from 260.5 to 16.5 g O<sub>2</sub> g N<sup>-1</sup>. Thus microbes become less efficient at producing organic matter relative to their nutrient uptake as decomposition progresses.

The model is over-parameterized such that it is not possible to determine the exact value of any one of RQ, PR, or  $CN_{microbes}$  without fixing the values of the other two. However we can

calculate possible combinations of the three that would result in the observed  $NUE_R$  for a given date (Figure 6). We can then apply theoretical boundaries estimated from the literature for each parameter (dashed boxes, Figure 6). Cole and Pace (1995) estimated that PR should be between 0.28 and 0.43 suggesting conservative limits of 0.2 to 0.5. Dilly (2001) measured RQ values of approximately 0.5 to 1 during basal metabolism on soil microbial communities from an unmanaged region in northern Germany suggesting values of 0.5 to 1.25. Finally, during decomposition leaves are initially colonized by fungi (Gulis and Suberkropp, 2003) which tend to have a C:N of approximately 8 (Cross and others, 2005). Later in decomposition bacteria (C:N  $\sim$  5; Cross and others 2005) colonize the substrate as fungal biomass decreases. Since fungi maintain a much higher standing crop than bacteria throughout decomposition, we can conservatively assume that CN stays between 5 and 10 with values at the higher end of this range being more likely. Incorporating this temporal progression in CN values with Figure 6 suggests that PR and RQ both generally increase through time. However, in February the CN<sub>microbes</sub> necessary to keep RQ and PR within the theoretical limits becomes unrealistically low (~2.53) indicating that, although changes in  $NUE_R$  in the microbial community may occur, they alone cannot explain the differences in predicted and observed NO<sub>3</sub>-N uptake.

Over-prediction at the beginning of autumn may also be caused by shifts in the sources of N used to supply microbial assimilation. We assumed that all nutrient demand was satisfied by water column N and ignored substrate N mineralized during decomposition. Early microbial reliance on substrate-derived N would have caused lower than expected N uptake and, as a result, high NUE<sub>R</sub> at the beginning of the season. As decomposition progresses through autumn, litter substrate quality should decline as heterotrophs preferentially assimilate labile detrital material and associated organic N supplies decline. Accordingly, leaf biofilms should switch to water column N to satisfy their nutritional requirements. As this shift occurs on individual leaves and the proportion of new litterfall (greater substrate N) decreases relative to the total CBOM standing crop, our predictions would more closely match observed patterns.

If modeled uptake is the sum of water column uptake  $(U_{calc})$  and uptake from leaf substrate, we can adjust equation (8) to solve for the C:N of leaf substrate ( $CN_{substrate}$ ) required to equate observed and modeled demand:

$$CN_{substrate} = \frac{R_{area} * \frac{12}{32} * PR * RQ}{U_{calc} - U_{observed}}$$
(9)

Note that we are again assuming our previous values of RQ, PR, and CN<sub>microbes</sub> from the literature. CN<sub>substrate</sub> can be positive only if uptake is over-predicted by the model. This occurred on the first four dates when input of new leaf material was most prominent. For these dates we calculated values of 5, 5, 6.37 and 18.45 for CN<sub>substrate</sub>. These values are much lower than those measured for litterfall worldwide  $(66.2 \pm 6.3)$  or for litterfall in temperate forests  $(58.4 \pm 3.7)$ ; McGroddy and others 2004). In addition, the lowest C:N value we measured for any species in litterfall in this study was 24 (data not shown), still higher than all of our estimated CN<sub>substrate</sub> values. Thus these estimates appear to represent a labile portion of overall leaf biomass. Since we assumed all mineralized N is used before any uptake from the water column occurs, these estimates represent a lower limit to CN<sub>substrate</sub> (i.e., highest required food quality). Higher CN<sub>substrate</sub> values would result from a higher proportion of uptake from the water column or from higher values of CN<sub>microbes</sub>. However the general pattern of CN<sub>substrate</sub> increasing through time supports our hypothesis that low C:N portions of the leaf are consumed first. Results from other litter decay studies lend support to this scenario. Melillo and others (1989) observed a decrease in acid-soluble carbohydrates (i.e., labile substrate) during the first stages of terrestrial litter decomposition with later stages characterized by an abundance of refractory lignified material. Similar patterns have been observed in aquatic systems (Webster and Benfield 1986). Melillo and others (1989) also observed an initial increase in absolute amounts of N associated with leaf material at the beginning of decay even while absolute amounts of C were declining. This occurs only with net uptake of N. The same pattern has been observed in streams (Triska and Sedell 1976). While we did not observe any N uptake at the beginning of decomposition, our observed lack of demand for NO<sub>3</sub>-N does not necessarily imply a lack of demand for N. It is likely, therefore, that net accumulation of N in CBOM occurred at the onset of decomposition as a combined result of retention of substrate-derived N and limited uptake from the water column. Since substrate N availability does not explain under-prediction of demand on the last two dates, we suspect that differences on these dates were due to higher NUE<sub>R</sub> associated with substrates more dominated by microbial biomass later in leaf decay (Kaushik and Hynes 1971, Suberkropp and Klug 1976).

The tradeoff between dissolved and particulate nutrient sources observed in this research is particularly interesting and is suggestive of a continuum of resource supply between terrestrial and marine ecosystems. Nutrient supply in terrestrial environments is constrained by the retention and breakdown of particulate nutrient sources resulting in distinct patterns of organismal carbon:nutrient ratios and nutrient retention strategies (McGroddy and others 2004). In contrast, ocean currents and upwelling provide mixing of dissolved nutrient forms that maintains strongly consistent algal C:N:P ratios (Redfield 1958). Our results suggest that decomposition in headwater streams is largely controlled by similar mechanisms as decomposition in terrestrial environments (i.e., initial N increase, microbial processing, substrate quality limitation), but that microbes in streams may utilize a well-mixed, dissolved nutrient sources changes downstream (Vannote 1980) it is likely that nutrient cycling and nutrient retention strategies adjust accordingly and tend toward marine systems.

# **Literature Cited**

- American Public Health Association. American Water Works Association, Water Environment Federation. 1998. Standard methods for the examination of water and wastewater, 20<sup>th</sup> Ed. CL. S. Clesceri, A.E. Greenberg, A.D. Eaton, eds., Method 5310C. Total Organic Carbon (TOC) Wet-oxidation Method, pp. 5-17-5-26. APHA, Washington, DC.
- Benfield EF, Webster JR, Tank JL, Hutchens JJ. 2001. Long-term patterns in leaf breakdown in streams in response to watershed logging. Internat. Rev. Hydrobiol. 86:467-474.
- Bott TL. 1996. Primary productivity and community respiration. In: Methods of Stream Ecology. Hauer FR, Lamberti GA, editors. San Diego: Academic Press. p533–556.
- Clinton BD, Vose JM. 1996. Effects of *Rhododendron maximum* L. on *Acer rubrum* L. seedling establishment. Castanea 61:38-45.
- Chapin FS. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11:233-260.
- Cole JJ, Pace ML. 1995. Bacterial secondary production in oxic and anoxic freshwaters. Limnology and Oceanography 40:1019–1027.
- Cross WF, Benstead JP, Frost PC, Thomas SA. 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50:1895-1912.
- Dilly O. 2001. Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter. Soil Biology and Biochemistry 33:117-127.
- Earl SR, Valett HM, Webster JR. 2006. Nitrogen saturation in stream ecosystems. Ecology 87:3140-3151.
- Elser JJ, Dobberfuhl DR, Mackay NA, Schampel JH. 1996. Organism size, life history, and N:P stoichiometry: toward a unified view of cellular and ecosystem processes. Bioscience 46:674-684.
- Elwood JW, Mulholland PJ, Newbold JD. 1988. Microbial activity and phosphorus uptake on decomposing leaf detritus in a heterotrophic stream. Verh. Internat. Verein. Limnol. 23:1198-1208.
- England LE, Rosemond AD. 2004. Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. Freshwater Biology 49:721-734.
- Fellows CS, Valett HM, Dahm CN, Mulholland PJ, Thomas SA. 2006. Coupling nutrient uptake and energy flow in headwater streams. Ecosystems 9:788-804.

- Gulis V, Suberkropp K. 2002. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. Microbial Ecology 45:11-19.
- Gulis V, Suberkropp K. 2003. Leaf litter decomposition and microbial activity in nutrientenriched and unaltered reaches of a headwater stream. Freshwater Biology 48:123-134.
- Hall RO, Tank JL. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnology and Oceanography 48:1120-1128.
- Hancke K, Glud RN. 2004. Temperature effects on respiration and photosynthesis in three diatom-dominated benthic communities. Aquatic Microbial Ecology 37:265-281.
- Hauer FR, Hill WR. 1996. Temperature, Light, and Oxygen. In: Methods of Stream Ecology.Hauer FR, Lamberti GA, editors. San Diego: Academic Press. p93-106.
- Hedin LO, von Fischer JC, Ostrom NE, Kennedy BP, Brown MG, Robertson GP. 1998. Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces. Ecology 79:684-703.
- Kaushik, NK, Hynes HBN. 1971. The fate of dead leaves that fall into streams. Archiv Fur Hydrobiologie 68: 465-515.
- Kilpatrick FA, Cobb ED. 1985. Measurement of discharge using tracers: U.S. Geological Survey, Techniques of Water Resources Investigations, Book 3, Chapter A16, 52 pp.
- Kirchman DL, Wheeler PA. 1998. Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Arctic Pacific. Deep-Sea Research 45:347-365.
- Liu HS, Li LH, Han XG, Huang JH, Sun JX, Wang HY. 2006. Respiratory substrate availability plays a crucial role in the response of soil respiration to environmental factors. Applied Soil Ecology 32:284-292.
- McGroddy ME, Daufresne T, Hedin LO. 2004. Scaling of C:N:P stoichiometry in forests worldwide: Implications of terrestrial Redfield-type ratios. Ecology 85:2390-2401.
- Melillo, JM, Aber JD, Linkins AE, Ricca A, Fry B, Nadelhoffer KJ. 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Plant and Soil 115:189-198.
- Menzel DW, Vacarro RF. 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnology and Oceanography 9:138–142.

- Mulholland PJ. 2004. The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. Biogeochemistry 70:403-426.
- Mulholland PJ, Newbold JD, Elwood JW, Webster JR. 1985. Phosphorus spiraling in a woodland stream: seasonal variations. Ecology 66:1012-1023.
- Redfield AC. 1958. The biological control of chemical factors in the environment. American Scientist 46:205-221.
- Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14:779–801.
- Stark JM, Hart SC. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385:61-64.
- Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton Univ. Press.
- Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. Journal of the North American Benthological Society 9:95-119.
- Suberkropp K, Klug MJ. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57:707-719.
- Tank JL, Webster JR, Benfield EF. 1993. Microbial respiration on decaying leaves and sticks in a southern Appalachian stream. Journal of the North American Benthological Society 12:394-405.
- Tank JL, Meyer JL, Sanzone DM, Mulholland PJ, Webster JR, Peterson BJ, Wollheim WM,
   Leonard NE. 2000. Analysis of nitrogen cycling in a forest stream during autumn using
   a <sup>15</sup>N-tracer addition. Limnology and Oceanography 45:1013-1029.
- Triska FJ, Sedell JR. 1976. Decomposition of four species of leaf litter in response to nitrate manipulation. Ecology 57:783-792.
- USEPA. 1997. Method 349.0. Determination of ammonia in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis. Zhang, J.Z., P.B. Ortner, C.J. Fischer, L.D. Moore. In Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices – 2<sup>nd</sup> Edition. EPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, Ohio.

- Valett HM, Crenshaw CL, Wagner PF. 2002. Stream nutrient uptake, forest succession, and biogeochemical theory. Ecology 83:2888-2901.
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37:130-137.
- Wallace JB, Eggert SL, Meyer JL, Webster JR. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102-104.
- Webster JR, Benfield EF. 1986. Vascular Plant Breakdown in Freshwater System. Annual Review of Ecology and Systematics. 17:567-594.
- Webster JR, Golladay SW, Benfield EF, D'Angelo DJ, Peters GT. 1990. Effects of forest disturbance on particulate organic matter budgets of small streams. J. N. Am. Benthol. Soc. 9:120-140.
- Webster JR, Benfield EF, Ehrman TP, Schaeffer MA, Tank JL, Hutchens JJ, D'Angelo DJ.
  1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. Freshwater Biology 41:687-705.
- Webster JR, Benfield EF, Hutchens JJ, Tank JL, Golladay SW, Adams JC. 2001a. Do leaf breakdown rates actually measure leaf disappearance from streams? International Review of Hydrobiology 86:417-427.
- Webster JR, Mulholland PJ, Tank JL, Valett HM, Dodds WK, Peterson BJ, Bowden WB, Dahm CN, Findlay S, Gregory SV, Grimm NB, Hamilton SK, Johnson SL, Marti E, McDowell WH, Meyer JL, Morrall DD, Thomas SA, Wollheim WM. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. Freshwater Biology 48:1329-1352.
- Webster JR, Tank JL, Wallace JB, Meyer JL, Eggert SL, Ehrman TP, Ward BR, Bennett BL,
  Wagner PF, McTammany ME. 2001b. Effects of litter exclusion and wood removal on
  phosphorus and nitrogen retention in a forest stream. Verh. Int. Ver. Limnol. 27:1337–1340.
- Webster JR. 2007. Spiraling down the river continuum: stream ecology and the U-shaped curve.J. N. Am. Benthol. Soc. *In press*.
- Wood, E. D., F. A. J. Armstrong, and F. A. Richards. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. Journal of the Marine Biological Association of the United Kingdom 47:23–31.



**Figure 1:** Temporal patterns in organic matter standing crops and chemical parameters. OM data are means ( $\pm$  SE) for sampling dates. Different letters denote statistically significant differences across dates.



**Figure 2.** Relationship of  $v_f$  and U with OM standing crops. Left panels (A and C) are versus CBOM standing crop and right panels (B and D) are versus Total OM (FBOM + CBOM).



**Figure 3.** Leaf disk respiration rates over the course of litterfall and decomposition. A) Rates measured at laboratory temperature (~20°C). B) Rates after  $Q_{10}$  transformation with  $Q_{10}=2.5$ . Symbols (means ± 1 SE) are (•) Birch, (•) Tulip Poplar, ( $\mathbf{\nabla}$ ) Beech, ( $\Delta$ ) Oak, ( $\mathbf{\blacksquare}$ ) Rhododendron.



**Figure 4.** Measured (•) and model predicted (---) CBOM standing crops. Observed values are black dots (means  $\pm 1$  SE). Dashed line is modeled total stream CBOM standing crop.



**Figure 5.** Comparison of N demand predicted by the model and observed whole-stream demand. Arrows indicate progress through time. Dotted line indicates the 1:1 line.



**Figure 6.** Calculated values of the microbial production coefficient (PR), respiratory quotient (RQ), and microbial C:N (contours) that would have resulted in the observed rates of uptake for each date. Values were estimated for each date on which uptake was observed. Boxes indicate conservative theoretical boundaries for RQ and PR based on published values.

Date	Discharge	Temp	NO <sub>3</sub> -N	DOC	Sw	¥	5
	ل م_	ຸວ	ug L <sup>-1</sup>	mg L <sup>-1</sup>	E	mm min <sup>-1</sup>	g N m⁻² d⁻¹
October 1, 2005	6.2	16	37 ± 0.3 (20)	0.507 ± 0.015 (10)	ı	00.00	0.0000
October 15, 2005	6.2	14.5	15 ± 0.6 (20)	0.576 ± 0.013 (11)	ı	00.0	0.0000
October 29, 2005	5.6	6	13 ± 0.2 (20)	0.435 ± 0.010 (11)	506	0.22	0.0041
November 21, 2005	5.3	9.7	1 ± 0.1 (21)	0.888 ± 0.062 (15)	158	0.66	0.0009
December 3, 2005	3.1	5.6	7 ± 0.4 (21)	0.338 ± 0.051 (12)	260	0.67	0.0071
January 12, 2006	7.9	7.9	22 ± 0.2 (19)	0.289 ± 0.054 (10)	300	0.44	0.0140
February 18, 2006	14.7	6.4	57 ± 3.1 (17)	NA	708	0.35	0.0291
Data are single measu parentheses are the m	tres for discharge umber of samples.	and temperat	ure. Data are m	ean values $\pm I$ SE for .	chemical c	onstituents. Nu	mbers in

sampling date.
n each
variables of
ysico-chemical
le 1. Ph
Tab

	Initial	Breakdown			Litter	fall rates			
Leaf Species	standing stock	rate	1-Oct	8-Oct	15-Oct	29-Oct	19-Nov	3-Dec	Total input
	g AFDM m <sup>-2</sup>	degree day <sup>_1</sup>		g AF	'DM m <sup>-2</sup> sir	nce last col	lection		g AFDM m <sup>-2</sup>
Tulip Poplar	06.6	0.001486	10.4	8.1	17.0	17.8	13.1	1.6	68.0
Sweet Birch	12.79	0.000486	5.0	0.9	7.7	12.3	19.5	0.0	45.4
American Hornbeam	6.27	0.000486	1.7	2.7	2.4	11.7	4.1	0.2	22.8
Hickory	4.43	0.000784	1.1	0.0	0.2	4.0	8.8	3.2	17.3
Chestnut	2.37	0.000595	0.0	0.2	0.0	0.0	7.9	0.2	8.4
Beech	4.04	0.000595	0.1	0.1	0.0	0.8	12.7	0.5	14.3
Red Maple	1.22	0.001486	0.0	0.5	0.0	0.5	4.9	0.4	6.4
Oak	7.87	0.001081	0.6	0.0	0.0	0.8	28.1	3.8	33.3
Eastern Hemlock	5.71	0.000270	11.3	0.1	1.1	0.5	2.4	1.7	17.0
Rhododendron	2.65	0.000622	2.6	0.0	2.5	2.0	3.5	0.0	10.7
White Pine	1.31	0.000743	2.0	0.0	0.0	0.0	3.1	0.3	5.4
Misc Leaves	4.43	0.000784	13.2	0.1	0.3	1.7	5.0	0.6	20.8
Initial standing crop is 1 buckets since the last co	he CBOM crop mode Ilection date. The fir.	led on day 1 of the st collection date (	e model. L October 1	itterfall ra <sup>st</sup> ) was assi	tes are the a umed to have	verage input e occurred 3(	of litterfall n ) days after ti	ieasured in he last colli	the collection ection.

ps	
ro	
し 20	
ш.	
p	
taı	
50	
ing	
e	
oq	
Ш	
ш.	
ba	
use	
S	
te	
ne	
rar	
pai	
n]	
M	
ğ	
a,	
οre	
l p	
an	
III	
rfa	
tte	
Ľ	
6 7	
pld	
[a	
r ,	