This thesis reports the results of a thirteen-day quantitative research project that assessed the relationship between leaf stoichiometry and nutrient uptake with the use of microcosms.
THE INFLUENCE OF THE STOICHIOMETRY AND DECOMPOSITION OF COTTONWOOD AND SUGAR MAPLE LEAVES ON NUTRIENT UPTAKE IN MICROCOSMS

Madeleine V. Mahan

Nutrient removal from forested woodland streams is influenced by microbial activity. Microorganisms fulfill their elemental needs by removing nutrients from organic substrate as well as removing dissolved inorganic nutrients directly from stream water. How microorganisms acquire nutrients suggests that variation in leaf litter stoichiometry may affect nutrient uptake from stream water. In this study, sugar maple and cottonwood leaves were isolated in microcosms containing nutrient-enriched stream water to assess the potential for using stoichiometric theory to estimate nutrient uptake over time. Leaves were in the early stages of decomposition and were isolated in microcosms for a period of two hours. I hypothesized that leaf stoichiometry would influence nutrient uptake, resulting in greater nitrogen uptake in species with a low N:P ratios (sugar maple) and greater uptake of phosphorus in species with a high N:P ratios (cottonwood). I also hypothesized that this relationship would continue as the chemical composition of leaves was altered by decomposition.

As predicted, microbes on sugar maple leaves took up more N from the microcosm over the course of the incubation period and cottonwood microbes took up more P, but as the leaves decomposed this distinction became less clear. In this experiment the microbial colonization of leaves had a significant effect on total leaf stoichiometry, which became more pronounced by
the end of the sampling period. Additionally, there was an observed contribution of oxygen to
the microcosms during the isolation period, suggesting the presence of flocculant algae.

Although data are accurate, confounding factors limited the visibility of stoichiometric
relationships, reducing the potential to validate whether organic matter stoichiometry could
serve as a predictor of nutrient uptake. My study suggests that with greater variable isolation,
this approach could be used to predict nutrient uptake based on the stoichiometric consumer-
resource imbalances in heterotrophic streams.
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THE INFLUENCE OF THE STOICHIOMETRY AND DECOMPOSITION OF COTTONWOOD
AND SUGAR MAPLE LEAVES ON NUTRIENT UPTAKE IN MICROCOSMS

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M. V. M.
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CHAPTER I
INTRODUCTION

**Nutrient Uptake in Streams**

The uptake and transformation of nutrients in stream ecosystems are commonly driven by autotrophic (algae) and heterotrophic (bacteria and fungi) biological processes (Earl, 2004). The biological uptake of nutrients and incorporation into new tissue is also referred to as immobilization (Allan and Castillo, 2007a). Immobilization results in the conversion of dissolved inorganic nutrients to an organic state. Nutrients stay in an organic state until the organism dies and the decomposition process, otherwise known as mineralization, releases the nutrients back into the water column as inorganic constituents (Allan and Castillo, 2007a). How quickly nutrients cycle is determined by three variables: 1) the rate at which nutrients are taken up by algae and microbes per unit area of streambed, 2) the demand for nutrients within the stream, and 3) stream size and water velocity (Webster and Ehrman, 1996). In natural streams, which are commonly nutrient limited, more productive systems have faster uptake rates (Hoellein et al., 2007). Though nitrogen and phosphorus uptake occur in tandem, studies on nutrient uptake (NU) in streams have commonly focused on the movement of a single element (Mulholland and Webster, 2010, Haggard et al., 2005, and Newbold et al., 2006) which neglects the relationship between the dual uptake of N and P.
The individual properties of nutrients also influence the rate at which they cycle through streams. For example, the uptake of N-NH₄⁺ is often higher than that of N-NO₃⁻ because N-NH₄⁺ is a more basic form of N, meaning it is easier to process biologically, and is therefore more readily used by stream organisms (Simon et al., 2005). Overall, the combined forms of N (NH₄⁺ and NO₃⁻) are taken up in greater amounts than P-PO₄⁻ to satisfy the stoichiometric requirements of microorganisms. When assessing uptake, abiotic factors must also be taken into consideration. For example, adsorption differentially affects separate forms of N and P. Adsorption processes have a strong influence on P-PO₄⁻, less of an effect on N-NH₄⁺, and almost no influence on N-NO₃⁻ (Allan et al., 2007).

Metabolism and nutrient uptake

The uptake of nutrients in streams is driven by metabolic processes such as respiration and primary production (Fellows et al., 2006, Valett et al., 2008, Gibson and O’Reilly, 2012). The efficiency with which nutrients are processed varies from stream to stream. Variation in energy inputs, processes of decay and consumption, the presence of organic carbon, stream size, and the characteristics of the surrounding watershed all contribute to the rate of nutrient uptake (Allan and Castillo, 2007b). Metabolic processes vary along the river continuum. The ratio of primary production (p) to respiration (r) is used as a measurement of the relative importance of internal/external organic carbon inputs. This ratio can be used to measure the changes in stream metabolism (Allan and Castillo, 2007b). For example, in headwater streams, the overhanging tree canopy affects stream metabolism by contributing large inputs of organic matter while limiting sunlight. These conditions result in a p/r ratio of less than 1, meaning low-order streams rely heavily on external carbon sources to sustain respiration. However, in mid-
sized streams, the canopy no longer shades the reach which encourages the autochthonous production of carbon. These conditions result in a p/r ratio greater than 1, meaning that respiration needs are satisfied by the internal production of organic matter (Allan and Castillo, 2007b).

Within a stream, nutrients are taken up at the cellular level by microbes (fungi and bacteria) and algae. The activity of each of these groups is measured differently, with community respiration representing microbial activity and gross primary production representing the activity of algae. Both of these measurements describe how energy is transferred within a system; however, neither of these parameters may accurately represent the rate of nutrient uptake in the system. A study by Hoellein et al. (2009) comparing the uptake of NO$_3^-$, NH$_4^+$, and soluble reactive phosphorus (SRP) to the rate of autotrophic metabolism (gross primary production) and heterotrophic metabolism (community respiration) in a stream, found that community respiration alone significantly predicts nutrient uptake (though the relationship was not strong). Gross primary production proved to be a significant predictor of nutrient uptake only for NH$_4^+$, but with an R$^2$ value of 0.217, the relationship was weak. The potential for the use of gross primary production as an indicator of microbial activity was further diminished by its inability to significantly predict nutrient uptake for NO$_3^-$ and SRP. Community respiration significantly predicted uptake for NO$_3^-$, NH$_4^+$, and SRP but with the respective R$^2$'s of 0.186, 0.358, and 0.280 the potential for its use as a predictor of nutrient uptake is low. This study indicated that although microbial activity appears to be more important than algal activity in controlling nutrient uptake, metabolism (specifically community respiration) alone cannot explain minor changes in nutrient uptake. Looking more closely at microbial nutritional
requirements and the nutritional composition of an organic matter substrate (leaf species) may illustrate more clearly what controls nutrient uptake.

**Microbial stoichiometry and nutrient uptake**

The uptake of nutrients in streams can be framed by stoichiometric theory, or how the balance of chemical elements is maintained in ecological interactions. Elemental imbalances between nutrients available from organic matter and what is required by a consumer affects rates of growth, reproduction, and maintenance within the food chain (Frost and Elser, 2002, Sterner, 1997). When allochthonous material enters a stream it is processed and used by decomposer microorganisms and detritivores (Suberkropp and Chauvet, 1995). These decomposer microorganisms have a fixed elemental signature which requires that they acquire nutrients in a specific ratio (C:N:P = 646:31:1; Webster et al., 2009). To fulfill microbial elemental needs, microorganisms supplement their nutrient supply by removing what they need directly from the dissolved inorganic nutrients in the water column (Suberkropp and Chauvet, 1995, Stelzer et al., 2003). The fact that microbes automatically remove inorganic nutrients from the water when there is a stoichiometric imbalance between their bodies and the organic substrate they are living on could make the stoichiometry of microbial substrate a good predictor for nutrient dynamics in the water column of heterotrophic streams. Autotrophs (algae) also contribute to the uptake of nutrients in the water column but their stoichiometry is more variable (C:N:P = 95:14:1 to 110:45:1; Hill et al., 2011), meaning that algal community molar ratio may not be a good predictor of nutrient uptake, which is consistent with the poor relationships between nutrient uptake and gross primary production (Hoellein et al., 2007).
Microbes, fungi in particular, are the most important biological drivers for detritus-associated nutrient uptake, which is dominant in forested headwater streams (Gulis et al., 2008, Suberkropp and Chauvet, 1995). Decomposition associated with microbial activity is responsible for up to 65% loss of leaf litter mass within the first 6 weeks in a stream (Baldy et al., 1995). Microbial community molar is C:N:P = 646:31:1 (Webster et al., 2009). The closer the microbial molar ratio is to the stoichiometry of stream substrate, the more likely it is that the substrate will serve as an appropriate microbial food source. Leaves have a higher nutrient content (a larger proportion of N and P related to C) than woody detritus, making leaves more suitable to supporting microbial activity than other allochthonous material. However, there is also stoichiometric variation among leaves; the C:N:P of a sugar maple leaf is 61:2:1 (Ostrofsky, 1997) and the C:N:P of a cottonwood leaf is 1260:40:1 (Tibbets and Molles, 2005). The higher the C:N:P ratio of organic matter, or the farther away it is from the microbial community stoichiometry, the lower the amount of potential nutrient uptake from the organic matter substrate, and the greater the nutrient needs of the microbial community.

The stoichiometric imbalance between microbes and their organic matter substrate drives microbes to supplement their elemental needs with dissolved inorganic nutrients from the water column. A study by Suberkropp and Chauvet (1995) comparing stream chemistry to the rate of leaf breakdown by fungi, found that when leaves residing in a stream with a low nutrient concentration were moved to a stream with a higher concentration of nutrients, the leaves were rapidly colonized by fungi. This study indicates that the nutrient content of water plays a significant role in the leaf breakdown by affecting the productivity of fungi. A more recent study by Schade et al. (2005) examined this relationship in greater detail and proposed
that the amount of N or P removed from the water column should be proportional to the stoichiometric imbalance between microbes and organic matter. Knowing that changes in the stoichiometry of organic matter should produce predictable shifts in nutrient uptake from the water column, stoichiometry may serve as an additional predictor for nutrient uptake than the traditional measurement of stream metabolism.

**Organic matter substrate and nutrient uptake**

Allochthonous materials, mainly leaves, make up the majority of the energy base in woodland streams (Minshall et al., 1983, Suberkropp and Chauvet, 1995). For this reason, the species composition of leaf litter and individual leaf stoichiometries may play an important role in determining nutrient uptake. The presence of leaves in streams is predominantly controlled by seasonality, with leaf inputs in streams running through deciduous forests increasing in late summer and peaking in the fall (Anderson and Sedell, 1979).

The moment allochthonous material enters a stream it begins to break down. For leaves, this process consists of three phases: 1) the initial leaching of soluble compounds, 2) a period of microbial decomposition and conditioning, and 3) invertebrate fragmentation (Webster and Benfield, 1986). As leaves decompose, they lose mass and nutrients. Though the nutrient content of the leaf is metabolized during this period, the presence of microbes on the leaf enhances the nutritional value of detritus. For this reason, leaves colonized in streams containing high concentrations of nutrients are expected to be a higher quality food source for invertebrate shredders due to the abundance of microbes on the leaves who are sustained by high-nutrient water (Suberkropp and Chauvet, 1995).
Although the rate of breakdown is affected by many factors such as water temperature, dissolved nutrient concentrations, and prior terrestrial decomposition or leaching, the greatest variation in the rate of decomposition within a stream is attributed to the unique stoichiometry of different leaf species (Baldy et al., 1995). Differences in the rate of breakdown between individual species have long been recognized (Petersen and Cummins, 1974 and Gessner and Chauvet, 1994), but it is unclear what impact their decomposition has on nutrient uptake from the water column and ecosystem function. For example, over time microbes should deplete the N concentration in a leaf and should consequently take up more N from the water column. The design of recent studies in natural streams has necessitated that all species of leaves be grouped together as coarse particulate organic matter to account for the compositional variability of organic matter (Lauridsen et al., 2012, Hoellein et al., 2009). This designation accurately captures the whole-reach processes but does not explain how much each leaf species contributes to nutrient uptake within the stream. In this study, I created an environment where individual leaf species were isolated, to more accurately assess the validity with which stoichiometric theory estimates nutrient uptake over time.

**Hypotheses**

1. I hypothesized that there would be greater nitrogen uptake in the presence of/associated with species with a low N:P (such as sugar maple, N:P=2:1). Similarly, I predicted there will be greater uptake of phosphorus in species with a high N:P (like cottonwood, N:P=40:1)
Leaves rapidly lose nutritional quality to chemical leaching when they enter a stream system. Leaves with higher N:P (cottonwood, 40:1) have more N to offer and will therefore lose more N to leaching and microbial activity, which will reduce the N:P of the leaves over time. Leaves with lower N:P (sugar maple, 2:1) leach a greater amount of P and have more taken up by microbes, which will cause N:P to rise over time. I hypothesized that when the N:P of each experimental leaf species is measured over time, the chemical composition of both species of leaves will approach each other. For the same reason, the nutrient uptake ratio (N:P) for each type of leaf should also become more similar as leaf N:P converges.
3. For leaves with high N:P I expected to see the increased uptake of P with greater metabolic rate. For leaves with low N:P I expected to see the increased uptake of nitrogen with greater metabolic rate.
CHAPTER II
METHODS

Study Site

This experiment was conducted at the Stream Research Facility at the University of Michigan Biological Station near Pellston, Michigan. Geologically, this area is characterized by post-glacial moraines and well-drained acidic sandy soils. Ground cover is predominantly secondary successional forest, due to widespread logging and burning in the early 20th century. Water at the Stream Research Facility is supplied by the East Branch of the Maple River, a relatively pristine, first-order river draining Douglas Lake (which is approximately 4.7 km upstream of the research facility). Initial stream water nutrient concentrations during the research period were typically around 20 µg/L for N-NH₄⁺, 30 µg/L N-NO₃⁻, and 10 µg/L for SRP. From where it originates, the East Branch of the Maple River flows almost entirely through protected UMBS nature preserve (Bambakidis 2009, Pan and Lowe 1995).

For this experiment, leaves were incubated in two low gradient, artificial streams. The stream were constructed with plastic roof gutters and fed continuously with water routed directly from the river, ensuring that temperature conditions mirrored the natural environment (21-23 °C).
Leaf Treatment

This study used leaves from sugar maple (*Acer saccharum*) and eastern cottonwood (*Populus deltoides*) trees to explore the relationship between stoichiometry and nutrient (N and P) uptake over time. The leaves used in this experiment were collected in Saratoga Springs, New York in fall 2011. This experiment necessitated that the leaves be collected shortly after they had fallen to maintain the chemistry and physical condition they would have if they had fallen...
off a branch and into a stream on their own. For this reason the leaves were collected ahead of
time at a more convenient location.

To accurately assess the impact that a specific form of organic substrate has on nutrient
removal as the material decomposes, my study isolated sugar maple and cottonwood leaves in
separate artificial streams. Dried sugar maple leaves were submerged in their respective stream
on July 4, 2012 and cottonwood submersion occurred on July 6. Both species of leaves were
incubated for a total of 13 days and were sampled on days 5 (cottonwood only), 6 (sugar maple
only), 9, and 13. Both experimental streams were kept shaded with a tarp to limit algae growth.

Table 1: Dates of leaf submersion and the sampling schedule

<table>
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<th>Leaf Species</th>
<th>Submerged</th>
<th>Trial 1 (SM: day 6, CW: day 5)</th>
<th>Trial 2 (day 9)</th>
<th>Trial 3 (day 13)</th>
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Microcosms containing a single leaf and stream water enriched for N-\(\text{NH}_4^+\), N-\(\text{NO}_3^-\), or SRP were used to measure community respiration and nutrient uptake for sugar maple and
cottonwood leaves (Hoellein et al., 2009). Stream water was first filtered with a 47mm glass
fiber filter (Pall A/E glass fiber filter, Ann Arbor, Michigan) and then enriched 10 µg/L above
ambient concentrations for either N-\(\text{NH}_4^+\), N-\(\text{NO}_3^-\), or SRP before being poured into microcosms
(200-260ml). To ensure that the presence of flocculant algae, which was common in the stream
water, did not dominate the leaf surface and affect dissolved oxygen, each leaf and stem was
wiped clean of algae by hand and rinsed in the artificial stream before being placed in a microcosm. For each nutrient treatment there were five replicate microcosms as well as three controls (filtered stream water mixtures only). A fourth set of microcosms with no nutrient treatment was filled with filtered stream water to measure metabolism in the absence of additional nutrients and to act as control.

Figure 2: Illustration of the assemblage of microcosms for each trial.

Microcosms were incubated for two hours in a 40 gallon pool built from cinder blocks and plastic sheeting. The pool was located at the end of an experimental stream to maintain ambient temperature and similar lights levels among all replicates. During the incubation period, samples were shaded by a tarp. Previous work (Hoellein et al., 2009) and preliminary studies indicated that two hour incubations were sufficient for observing changes in dissolved oxygen concentrations. During this time period measurable uptake occurs but the nutrients are not reduced to levels where they are limiting.
Dissolved Oxygen and Nutrients

After removal from the incubation pool, each microcosm was shaken to mix the water, and 60-ml of the sample was transferred into a 60-mL syringe. Oxygen levels in the syringe were fixed for later analysis with the addition of 200 µL of manganese sulfate and 200 µL of alkali-iodide-azide. Syringe tips were covered in Parafilm (Fisher Scientific, Pittsburg, Pennsylvania) to insure the samples were not exposed to additional oxygen, and syringes were refrigerated until dissolved oxygen was determined several hours later. An additional sample from each microcosm was filtered with a 25mm glass fiber filter (Pall A/E glass fiber filter, Ann Arbor, Michigan) into an acid-washed, 60-mL bottle. The samples were then frozen and later analyzed for N-NH₄⁺, N-NO₃⁻, or SRP with a segmented flow analyzer (Seal Analytical). The nutrient concentrations for the microcosms were determined by the UMBS lab according to the Standard Methods for the Examination of Water and Wastewater, 20th edition. The change in nutrient concentration for each sample was found by subtracting the nutrient concentrations in each microcosm from the average amount observed in the controls with the same type of water. From this, I found the change in nutrient mass which was then normalized by the mass of the leaf and the amount of time the leaf resided in the microcosm.

In the lab, the concentration of dissolved oxygen for each microcosm was established using the Winkler titration method after injecting syringes with 200µL of concentrated sulfuric acid and titrating with 0.025 N sodium thiosulfate dilution and applying the following calculation (Hauer and Lamberti, 2006):

$$\frac{mL \ of \ titrant \ used \times 0.025 \times 8000}{60.6 \times \left(\frac{60 - 0.6}{60}\right)} = mg \ O_2/L$$
The change in dissolved oxygen was calculated by subtracting the amount of oxygen in each microcosm from the average amount observed in the controls with the same type of water. The change in the amount of oxygen for each sample was then normalized to leaf mass and the amount of time the microcosm was sealed.

C:N:P of each sample leaf was measured at the UMBS analytical lab. In preparation, when sample leaves were removed from the microcosm, they were rinsed with filtered stream water, labeled and dried. Leaves were then baked overnight in an oven at 44 °C, weighed to find dry weight, and powdered. Because this study focused on the potential for stoichiometry to serve as a predictor of nutrient uptake and stems have higher carbon content than leaves (resulting in different stoichiometry) they were not included in the measurement of ash free dry mass or the C:N:P of the leaf.

A one-way analysis of variance (ANOVA) was used to compare the effects of nutrient addition on nutrient uptake for each leaf species across trials. If significance was found, a Tukey-Kramer comparison of means was used to identify which nutrient additions had a significant effect on uptake. The impact of nutrient addition on community respiration for each leaf species was assessed similarly with a one-way ANOVA and a Tukey-Kramer comparison of means when applicable.

In addition, the potential for the use of leaf stoichiometry as a predictor for nutrient uptake was assessed with a multiple regression model, comparing nutrient uptake to community respiration and leaf N:P for each species, across all trials. To further assess this claim, changes in leaf N:P for across trials were assessed with a one-way ANOVA, which was followed by a Tukey-Kramer comparison of means.
CHAPTER III

RESULTS

**Nutrient Uptake**

The greatest difference in nutrient uptake (Fig. 3) occurred in trial 1 for N-NH₄⁺ and SRP, with both sugar maple and cottonwood microcosms having rates of nutrient uptake which were significantly different than uptake in the other trials. In trial 1, nutrient uptake for sugar maple was significantly greater than nutrient uptake in trial 3 for N-NH₄⁺ and SRP and nutrient uptake of N-NH₄⁺ and SRP during trial 1 of cottonwood was found to be statistically different from nutrient uptake in trials 2 and 3. Excluding N-NO₃⁻ uptake for sugar maple in trial 2, nutrient uptake for both leaf species were typically very similar for each nutrient addition during trials 2 and 3.
Figure 3: Mean (±SE) uptake of (A) nitrate (N-NO$_3^-$), (B) ammonium (N-NH$_4^+$), and (C) soluble reactive phosphorus (SRP) for sugar maple and cottonwood microcosms in an experimental stream. Bars with the same letters indicate no significant difference between trials for that leaf type. Lower case letters correspond to the statistical relationship between sugar maple trials and upper case letters correspond to the statistical relationship between cottonwood trials. N.S. represents that there is no significant relationship between any of the bars for that leaf type. Trial 1 for sugar maple leaves occurred on day 6 of decomposition and trial 1 for cottonwood occurred on day 5. Trials 2 and 3 occurred on the same day in the decomposition process for both leaf species.
Nutrient addition and metabolism

The addition of nutrient-enriched water to microcosms had a limited effect on microbial activity. The only significant difference in community respiration related to nutrient addition occurred in sugar maple microcosms in trial 1, with additional N-NO\textsubscript{3}\textsuperscript{-} generating significantly greater microbial activity than microcosms with additional N-NH\textsubscript{4}\textsuperscript{+} or SRP, though there was no significant difference between N-NO\textsubscript{3}\textsuperscript{-} and control microcosms. For trials 2 and 3, there was no significant difference in community respiration for either leaf species. Negative community respiration values were not included in this analysis (Fig. 4) because gains in O\textsubscript{2} indicated the dominance of algal activity over microbes in the microcosm and did not allow me to assess the relationship between microbial activity and nutrient uptake. The following five microcosms experienced a gain in O\textsubscript{2} over the incubation period: 1) Trial 1- cottonwood leaf, N-NO\textsubscript{3}\textsuperscript{-} treatment, 2) Trial 2- cottonwood leaf, N-NO\textsubscript{3}\textsuperscript{-} treatment, 3) Trial 2- cottonwood leaf, no nutrient treatment, 4) Trial 2- maple leaf, no nutrient treatment, 5) Trial 3- maple leaf, no nutrient treatment.
Figure 4: Mean (±SE) uptake of O$_2$ (mg hr$^{-1}$ g$^{-1}$) on (A) day 5 (for cottonwood) and day 6 (for sugar maple), (B) day 9, and (C) day 13 for N-NO$_3^-$, N-NH$_4^+$, SRP treatments in sugar maple and cottonwood microcosms. Bars with the same letters are not significantly different among nutrient addition type. Lower case letters correspond to the statistical relationship between sugar maple trials and upper case letters correspond to the statistical relationship between cottonwood trials. N.S. represents that there is no significant relationship between any of the bars for that leaf type.
There was no significant relationship between nutrient uptake, community respiration, and N:P between trials and leaf species. This result was influenced by leaf N:P dynamics, which did not respond as hypothesized over the course of the experiment. N:P of sugar maple increased from trial 1 to trial 3 while the N:P of cottonwood leaves remained relatively the same (Fig. 5). Sugar maple leaves experienced significant change in N:P (ANOVA, P < 0.0001) with leaves in trial 3 having significantly greater N:P values than leaves in trials 1 and 2. There was no significant change in cottonwood leaf N:P across the trials.

Figure 5: Mean (±SE) of leaf stoichiometry from trial 1 (day 5) to trial 3 (day 13). Points with the same letters are not significantly different among trials. N.S. represents that there is no significant difference between any of the trials for that leaf type.
CHAPTER IV
DISCUSSION

Nutrient Uptake

Based on the relationship between the expected N:P of leaf species (sugar maple, 2:1 and cottonwood, 40:1) and microbial communities (30:1) I expected to see greater uptake of N-NO$_3^-$ and N-NH$_4^+$ in microcosms with sugar maple leaves and greater uptake of SRP in microcosms containing cottonwood leaves (hypothesis 1). Data on nutrient uptake reflect this hypothesis, confirming the presence of a basic stoichiometric relationship between the nutritional compositions of substrate and coupled nutrient uptake (Fig. 3). For N-NH$_4^+$ and SRP, nutrient uptake for both leaf species in trial 1 was significantly higher than nutrient uptake in trial 3 but there was no difference in nutrient uptake between trial 2 and trial 3 for either leaf species in N-NH$_4^+$ or SRP microcosms. As the leaves decomposed I had expected nutrient uptake to decrease but the lack of significant difference between trials 2 and 3 suggests significant nutrient uptake occurred more rapidly than expected. Though not statistically significant, the trend of the data suggests that earlier in the decomposition process there would be greater difference in the uptake of at least N-NH$_4^+$ and SRP between species, with greater uptake of N-NH$_4^+$ by sugar maple and greater SRP uptake by cottonwood (which is consistent with hypothesis 1).
Further variation in nutrient uptake occurred in the N-NH₄⁺ addition for cottonwood leaves in trial 1. All five replicate microcosms showed negative nutrient uptake for this trial, which to the best of my knowledge should be attributed to chemical leaching. Another data anomaly includes an increase in the uptake of N-NO₃⁻ for sugar maple microcosms in trial 2. It seems unusual for an increase in the uptake of N-NO₃⁻ not to be accompanied by an increase in the uptake of N-NH₄⁺. N-NH4⁺ is a more basic form of N, meaning it is easier to process, and is therefore more readily used by stream organisms (Simon et al., 2005). If microbes on sugar maple leaves required additional N, it would make sense that they would take up equally as much N-NH₄⁺, if not more, as they would N-NO⁻₃. Additionally, it should be noted that the standard error for nutrient uptake in NO₃⁻ samples was relatively high (SE ± 1.1 μg hr⁻¹ g⁻¹), so while the results were statistically significant there was variation among samples.

Community Respiration

Within trials, nutrient addition type had little effect on community respiration and the differences that were observed, suggested the influence of unanticipated conditions within the microcosm (Fig. 4). Aside from a lack of observed statistical significance, additional discrepancies with community respiration data concern negative values for change in O₂ during the incubation process. Several sample microcosms, as well as control samples, recorded a net increase in O₂. To achieve an increase in O₂ concentration, these particular samples may have been dominated by algal activity as opposed to microbial. During the experiment, visible algae were removed from the surface of the leaf before the leaf was placed in a microcosm but any increase in O₂ suggests that some amount of algae was included in the incubation. All samples with negative change in O₂ were removed from the body of data which was statistically analyzed. However,
increasing O₂ values suggest that even though the majority of samples were dominated by microbial activity, residual algae likely had some measure of impact on community respiration in all microcosms. For this reason, community respiration data should be considered an underestimation.

Leaf stoichiometry

Based on the stoichiometric relationship between microbial nutritional requirements and the known N:P of each leaf species, I hypothesized that as N and P were taken up from the leaves by microbes, the nutritional ratio of each species would become more similar (meaning that the N:P of cottonwood would lower and the N:P of maple would rise). However, at trial 1 (day 5), average leaf N:P of both species was nearly identical (sugar maple, N:P of 55.5, SE± 4.6 and cottonwood, N:P of 54.5, SE± 5.5) meaning that the N:P of both species had risen since placement in the experimental streams (Fig. 5). Average leaf N:P of both species continued to increase over the course of the trials though the only significant change in leaf N:P was observed in sugar maple leaves (average leaf N:P in trial 3 was significantly larger than N:P of trials 1 and 2).

I interpreted the divergence from the expected change in N:P to be a result of the incorporation of colonizing microbial biomass. Since microbial communities may develop internal as well as surface populations, when the leaf was wiped clear of algae it appears that microbial biomass remained intact (Beattie and Lindow, 1999). The microbial molar (30:1) is high in N, and the presence of microbial bodies could influence the overall concentration of N within the leaf, causing the ratio of N:P to increase with continued microbial activity. This relationship is best explained by the observed lack of change in N:P of cottonwood leaves. I had
anticipated a decrease in average N:P as microbes removed N from cottonwood leaves.

However, data show that cottonwood leaves gained N over the course of the experiment (Table 2). The addition of microbial N could have masked the hypothesized drop in leaf N:P, resulting in an average N:P that was apparently unchanging.

Table 2: Average change in nutritional composition for leaves across trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>%C</th>
<th>SE</th>
<th>%N</th>
<th>SE</th>
<th>%P</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41.532</td>
<td>0.569</td>
<td>0.905</td>
<td>0.047</td>
<td>0.037</td>
<td>0.004</td>
</tr>
<tr>
<td>2</td>
<td>42.079</td>
<td>0.432</td>
<td>1.014</td>
<td>0.061</td>
<td>0.035</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>41.08</td>
<td>0.387</td>
<td>1.105</td>
<td>0.046</td>
<td>0.028</td>
<td>0.003</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial</th>
<th>%C</th>
<th>SE</th>
<th>%N</th>
<th>SE</th>
<th>%P</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43.916</td>
<td>0.264</td>
<td>1.250</td>
<td>0.043</td>
<td>0.054</td>
<td>0.006</td>
</tr>
<tr>
<td>2</td>
<td>43.384</td>
<td>0.272</td>
<td>1.388</td>
<td>0.050</td>
<td>0.054</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>42.443</td>
<td>0.315</td>
<td>1.459</td>
<td>0.048</td>
<td>0.052</td>
<td>0.003</td>
</tr>
</tbody>
</table>

While the N:P of sugar maple leaves rose as expected, the influence of microbial colonization may be visible in the changing percentages of N and P (Table 2). According to the original hypothesis (hypothesis 2), rising N:P in sugar maple leaves was expected to result from a reduction in P. However, data showed that while P was removed from leaves over time, N was also gained. This gain potentially indicates the increased presence of microbial bodies within the leaf.
Validity of stoichiometric theory and implications for future research

With the use of microcosms, my study was able to isolate components of the system enough to observe differential uptake of N and P for leaves with N:P above and below that of microbial N:P (hypothesis 1) but this was the extent of my ability to support stoichiometric theory. Of my original hypotheses, differences in data were not significant enough to prove either hypothesis 2 or 3. The application of stoichiometric theory to the prediction of nutrient uptake in streams is supported by the known chemical composition of organisms, research on the effects of elemental imbalances within the food chain, and the knowledge of microbial acquisition of nutrients. This being said, recent studies in natural streams have had difficulty developing experiments allowing them to observe stoichiometric balance (Marti et al., 2009 and Simon et al., 2005). Marti et al. (2009) suggest that the reach of their study (which varied in geology and climate) was too broad to capture the fine-scale relationships controlling nutrient uptake.

Similarly to research in natural streams, in my study the visibility of stoichiometric relationships was limited by unanticipated variables. I hypothesize that the two main confounding factors were the presence of algae in microcosms and the contribution of microbial N:P to leaf stoichiometry.

Algae

The east branch of the Maple River is characterized by limited light and high input of organic materials, conditions which discourage algal growth (Gulis et al., 2008, Suberkropp and Chauvet, 1995). Typically, algal populations are more successful in mid-order streams that receive plenty of sunlight and have limited organic input (Vannote et al., 1980). In an effort to
discourage algal growth, leaves were kept shaded by a tarp for the duration of their time in the stream as well as during the microcosm incubation period. In addition to being in a light-limited environment, the stream was filled with leaves to mimic high organic input. Regardless of these precautions, there was continuous algal input via the stream water, and the algal population succeeded in colonizing the experimental stream. There is little potential to include algae in stoichiometric balances because algal C:N:P ranges from 95:14:1 to 110:45:1 (Hill et al., 2011). Unless the algal stoichiometry is restricted with the identification of specific species and algal biomass is determined, algal nutrient uptake remains relatively unpredictable. This variability is not conducive to the application of stoichiometric theory.

Continued research in this area would be improved by the exclusion of algae from microcosms. Due to the difficulty I encountered removing visible algae by hand, replications of this study may have greater success if they are able to limit the light source. In my study, the experimental streams were shaded but sunlight was able to penetrate the tarp. A light-free experiment design may help limit the influence of algae.

_Microbial nutrients_

As leaves decompose, they lose organic matter to leaching and nutrient uptake. As the leaf material loses its original nutrients, the nutrients can remain on the substrate in the form of microbial biomass. Studies have acknowledged the impact microbial communities have on the increase of nutrient quality in decomposing leaves but have found it difficult to quantify microbial influence (Gulis and Suberkropp, 2003). Future research would benefit from a greater knowledge of microbial influence on leaf nutrient quality and the ability to distinguish the changing chemical composition of the leaf from microbial biomass.
**Experiment design**

Future research seeking to capture the relationship of coupled nutrient uptake may benefit from a modified sampling schedule. In an effort to avoid the influence of chemical leaching from leaves, this study began sampling on the fifth day of leaf incubation in the experimental streams. Though some cottonwood leaves were still leaching at this point, the greatest significant differences between leaf species for nutrient uptake and community respiration were found in trial 1. Beginning sampling earlier in the decomposition process may better capture the fine scale differences in nutrient processing between leaf species.

Water temperature may also help determine appropriate sampling dates. A prior experiment examining the effect of water temperature on leaf litter decomposition rates found that higher water temperature increased the rate of leaf litter mass loss and decomposition (Ibrahim et al., 2010). For this reason, water temperature and temporal variation in experiment design should be taken into consideration and sampling dates should begin earlier when working with streams with warmer water (such as the 21-23 °C stream water used in this experiment).

Due to the varied rates of decomposition for different leaf species, a longer sampling period may also improve results. By the end of the experiment (day 13), sugar maple leaves had become papery and delicate and it was clear that they were decomposing more rapidly than cottonwood leaves. Given the proper resources, it would be interesting to observe changes in nutrient uptake and community respiration from the time leaves are placed in the experimental stream until the microbial biomass has been mineralized. A large sampling period would cover
variation in leaf decomposition rates (as well as leaching periods) and would provide better insight on the process of microbial colonization.
REFERENCES


Bambakidis, T., 2009, Changes in Benthic Algal Community Structure Following an Unpredictable Stream-Wide Desiccation Event [Master’s Thesis]: Bowling Green State University, 64p.


