DETRITAL CARBON RESPONSE TO EXPERIMENTAL ENRICHMENT AND IMPACTS ON ASSOCIATED CONSUMERS IN A HEADWATER STREAM

by

Cynthia Janelle Tant

(under the direction of Amy D. Rosemond)

ABSTRACT

Nutrient enrichment of aquatic ecosystems is occurring globally as a result of mobilization of nitrogen and phosphorus from a variety of anthropogenic sources. Responses to enrichment in detritus-based systems are not as well documented as those in autotroph-based systems. This study examined the effects of enrichment on a Southern Appalachian headwater stream on detrital resources and associated consumers. Heterotrophic microbes, particularly fungi, may perform a keystone function in headwater streams by facilitating carbon and nutrient flow to consumers and in the processing of allochthonous organic matter. I assessed the effect of nutrient enrichment on the relative contribution of decomposers and detritivores. I also examined changes on different size fractions of organic matter resources with enrichment and relationships between microbial biomass and nutrient content. Application of the Hieber-Gessner model suggests that nutrient enrichment may cause shifts toward increasing fungal and shredder contribution to leaf litter breakdown, intensifying losses of carbon through both respiration and downstream transport. The response of CPOM (both maple and rhododendron

substrates) to enrichment was primarily positive, but the response of FBOM to enrichment was unexpectedly low. Nutrient enrichment increased fungal biomass and microbial respiration and decreased C:N and C:P on CPOM substrates, suggesting a general increase in resource quality. Estimates of TERs and comparison to associated organic matter resources in each stream suggest that enrichment may reduce P limitation for shredders while potentially increasing C limitation for collectors. In a laboratory setting, CPOM mass loss and FPOM production were highest in treatments with both fungi and elevated nutrients. Both the presence of fungi and exogenous nutrients affected the N content of CPOM, but only nutrients had an effect on P content. Although fungi increased the availability of FPOM, the combined effects of fungi and nutrients did not appear to trickle down to FPOM nutrient content during organic matter transformations. These results highlight potential mechanisms for food web shifts and pathways of carbon processing that may result from nutrient enrichment of detritus-based systems.

INDEX WORDS: Headwater stream, Detritus, Detritivore, Heterotrophic microbe, Fungi, Bacteria, Nitrogen, Phosphorus, Nutrient enrichment, *Pycnopsyche*, Coweeta, Southern Appalachian

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by

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Nutrient enrichment of aquatic ecosystems is a significant source of impairment to freshwater and coastal waters, accounting for > 50% of impairment in lakes and streams in the U.S (Carpenter et al. 1998). Excess nutrients in streams and rivers come from both agriculture, in the form of fertilizers and animal waste, and urban sources, as municipal waste and fertilizers (Carpenter et al. 1998). These sources of nitrogen (N) and phosphorus (P) impact systems in a variety of ways, including algal blooms, fish kills, and loss of species diversity (Carpenter et al. 1998). While many research and management efforts have focused on the effects of nutrients on autotrophic-based pathways in aquatic food webs (e.g., phytoplankton or periphyton-based) (Rosemond et al. 1993; Correll 1998; Miltner and Rankin 1998; Biggs 2000; Dodds and Welch 2000), much less is known about heterotrophic pathways, or the effects of nutrient enrichment on food webs that are primarily driven by allochthonous detrital inputs.

Many ecosystems are almost solely based on detritus, such as forested headwater streams (Fisher and Likens 1972; Wallace et al. 1997), where woody debris and leaf litter are the dominant energy sources. In fact, most stream ecosystems are net heterotrophic (Mulholland et al. 2002). Detrital resources in streams include both fine and coarse particulate organic matter and provide important food resources for a majority of consumers in detritus-based stream ecosystems. In general, C:N and C:P ratios, frequently used measures of quality, decrease with particle size (Stelzer et al. 2003).

While fungi dominate on coarse particulate organic matter (CPOM) (i.e. wood and leaves), bacteria have been shown to be the dominant microbes on fine benthic organic matter (FBOM) (Findlay et al. 2002). Fungi and bacteria also differ in elemental content and have lower carbon to nutrient ratios than particulate organic matter (Stelzer et al. 2003). Because detritus is a poor quality resource even with associated microbial biomass, nutrient enrichment can cause dramatic changes in detrital quality (Robinson and Gessner 2000; Grattan and Suberkropp 2001; Pascoal et al. 2005).

Heterotrophic microbes, particularly fungi, may perform a keystone function in headwater streams by facilitating carbon and nutrient flow to consumers and in the processing of allochthonous organic matter. Nutrients stimulate microbial activity (Suberkropp and Chauvet 1995; Ferreira et al. 2006; Baldy et al. 2007), with potential subsequent effects on organic matter resources, such as in affecting breakdown rates and detrital quality as well as potentially affecting higher trophic levels.

Project Overview

This study was conducted at the Coweeta Hydrologic Laboratory (CHL), operated by the USDA Forest Service, in the southern Appalachian mountains of North Carolina, USA. A paired watershed approach was used, in which a treatment stream was enriched with moderate levels of nitrogen and phosphorus for six years and compared to an adjacent, unaltered reference stream. Specific details of the nutrient enrichment can be found in (Gulis and Suberkropp 2003). This dissertation focused on years five and six of enrichment. Previous results showed a significant increase in the activity and production of heterotrophic microbes, particularly fungi, with enrichment (Gulis and Suberkropp 2003; Gulis et al. 2004; Gulis and Suberkropp 2004; Suberkropp et al. 2010), followed by

an increase in invertebrate production (Cross et al. 2006; Davis et al. 2010). These changes also impacted ecosystem function as measured by increased leaf litter breakdown rates during the first two years of enrichment (Greenwood et al. 2007) and decreases in leaf litter standing crop (Suberkropp et al. 2010). Additionally, organic matter budgets revealed an increase in FPOM export and ecosystem respiration with enrichment(Benstead et al. 2009). The purpose of this dissertation was to examine the response of organic matter resources and their associated consumers to longer-term, continuous nutrient enrichment.

Dissertation Objectives

Chapter 2: Nutrient enrichment alters the relative contribution of fungi, bacteria, and detritivores to leaf litter breakdown

Hieber and Gessner (2002) determined that shredders accounted for the largest portion of leaf mass loss in a moderately enriched German stream. Based on data for our study streams (Suberkropp et al. 2010), in which fungal response to nutrient enrichment has been profound, the relative importance of fungi to breakdown processes may increase in response to nutrient enrichment. The objectives of this study were to determine 1) the effect of longer-term nutrient enrichment on leaf litter breakdown of two leaf litter species of differing in leaf chemistry and 2) changes in relative contribution of microorganisms vs. macroinvertebrates to leaf litter breakdown as a result of nutrient enrichment.

Chapter 3: Differential effects of nutrient enrichment on fine and coarse organic matter fractions in a heterotrophic stream: implications for consumers and carbon dynamics

The objectives of this study were to quantify nutrient effects on the quality of FBOM and CPOM resources and examine the relationship between bacteria and fungi and their dominance on different substrate types. Relatively greater response was expected on CPOM due to initially higher C:N and C:P ratios. Additionally, a differential microbial response was expected due to dominance of bacteria on FPOM contrasted with dominance of fungi on CPOM (leaves).

Chapter 4: The essential role of aquatic fungi in nutrient-mediated organic matter transformations

Fungal mediated transformations of CPOM were examined under elevated nutrient concentrations to test whether fungal presence was critical to CPOM processing rates and FPOM production by an invertebrate consumer. Specifically, we measured CPOM mass loss and FPOM production in the presence and absence of elevated nutrients and fungi to determine the role of fungi in organic matter transformations.

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CHAPTER 2

NUTRIENT ENRICHMENT ALTERS THE RELATIVE CONTRIBUTION OF FUNGI, BACTERIA, AND DETRITIVORES TO LEAF LITTER BREAKDOWN[1]

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Abstract

Effects of nutrient enrichment are widespread and affect organisms and ecosystem function. Increasingly, nutrient enrichment is altering ecosystem processes, though the mechanisms are poorly understood. We determined whether nutrient enrichment changed the relative contribution of biotic vs. abiotic components to leaf litter breakdown. Within the biotic compartment, we quantified how the relative contribution of microbial decomposers vs. detritivores associated with leaf litter breakdown changed with increased nutrients using a dominant leaf species in many southern Appalachian headwater streams, *Rhododendron maximum* L. We measured leaf-associated shredder, fungal, and bacterial biomass, and estimated breakdown rates based on mass loss from leaf packs. Despite changes in biotic contributions to breakdown with enrichment, the cumulative contribution of biota to leaf litter breakdown did not exceed 35% in either stream, suggesting that abiotic factors had a greater effect than all of the combined biotic groups that we examined in these mountain streams. Changes in biomass of both fungi and shredding macroinvertebrates, however, appeared to drive increased rates of carbon loss over time with nutrient enrichment. Shredder contribution to breakdown increased over time and peaked at an earlier date in the enriched stream, reflecting increased rates of breakdown under enriched conditions. Fungal contribution to breakdown was highest during the period prior to shredder dominance and also peaked earlier in the enriched stream. Bacterial contribution was consistently low in both enriched and reference conditions. At similar stages of mass loss (~20%), fungi contributed as much as shredders to breakdown in the enriched stream. However, shredders dominated biological contributions to breakdown in the reference stream and at later stages of breakdown in both streams. Such changes in response to nutrient enrichment have the potential to alter quantities of carbon processed in stream

ecosystems at multiple scales and impact resource availability both locally and in downstream ecosystems.

Introduction

Primary consumers, both microbial and metazoan, play important roles in organic matter dynamics and energy flow in ecosystems (Cebrian 2004). In donor-controlled systems, detritivores and heterotrophic microbes contribute to breakdown of organic matter, which is critical to both carbon and nutrient cycling on global and local scales (Moore et al. 2004). In many stream ecosystems, heterotrophic sources of carbon in the form of allochthonous input of leaf litter provide much of the energy resources (Wallace et al. 1997).

Recent evidence indicates that detrital processing can increase with stream nutrient enrichment (Robinson and Gessner 2000; Pascoal et al. 2005), a widespread problem affecting both autotrophic and heterotrophic aquatic ecosystems (Dodds 2007; Dodds and Cole 2007). Nutrient enrichment has also been associated with increased biomass and production of macroinvertebrate consumers (Robinson and Gessner 2000; Rosemond et al. 2002; Chadwick and Huryn 2003) and heterotrophic microbes, particularly fungi (Suberkropp 1995; Suberkropp 1998). Although such studies have indicated that the dominant drivers of organic matter processing can increase with nutrient enrichment (Suberkropp and Klug 1980; Arsuffi and Suberkropp 1984), the relative contributions of microorganisms and macroinvertebrates to breakdown have rarely been measured in streams and the effects of nutrient enrichment on their relative contributions have not previously been determined (Hieber and Gessner 2002; Gulis and Suberkropp 2003a).

This study examines the response of decomposers and detritivores to a continuous, moderate addition of both nitrogen and phosphorus to a detritus-based headwater stream compared to a nearby reference stream. These streams were well-suited for testing our research question as previous studies found a significant increase in the activity and production of

heterotrophic microbes, particularly fungi, with nutrient enrichment on coarse particulate organic matter (CPOM)(Gulis et al. 2004; Gulis and Suberkropp 2004; Suberkropp et al. 2010), and an increase in invertebrate production (Cross et al. 2006; Davis et al. 2010). These changes have also impacted ecosystem function as measured by leaf litter breakdown rates over a two-year period (Greenwood et al. 2007). Other studies have shown that fungi play a dominant role in leaf litter breakdown (Suberkropp and Klug 1980; Arsuffi and Suberkropp 1984), but the relative contributions of microorganisms and macroinvertebrates to breakdown have rarely been measured in streams and the effects of nutrient enrichment on their relative contributions have not previously been determined (Hieber and Gessner 2002; Gulis and Suberkropp 2003a).

Hieber and Gessner (2002) developed a theoretical framework to partition the roles of microorganisms and metazoans in breakdown processes in streams. Using this framework, we determined the relative contribution of fungi, bacteria, and shredders to leaf litter breakdown and how contributions from each detritivore vs. decomposer group changed with increased nutrients. The objectives of this study were to determine 1) the effect of longer-term nutrient enrichment on breakdown of two leaf litter species differing in leaf chemistry and 2) whether nutrient enrichment increased the relative contribution of biotic vs. abiotic factors, and 3) whether the relative contribution of microorganisms vs. macroinvertebrates to leaf litter breakdown changed due to nutrient enrichment. Hieber and Gessner (2002) determined that shredders accounted for the largest portion of leaf mass loss in a moderately enriched German stream. Based on previous data from our study streams (Gulis and Suberkropp 2003b), in which fungi responded significantly to nutrient enrichment, we expected the relative importance of fungi to breakdown processes to increase in response to nutrient enrichment. Since previous studies showed that macroinvertebrate production also increased greatly in response to nutrient enrichment (Cross et

al. 2006; Davis et al. 2010), we predicted that nutrient enrichment would increase macroinvertebrate-mediated breakdown rates. Additionally, we expected that these increases in breakdown rates would be driven by increases in the relative contribution of microbes vs. macroinvertebrates, given the profound response of fungi to enrichment in this system (Suberkropp et al 2010).

Methods

This study was conducted within the framework of a long-term nutrient enrichment conducted in a paired watershed study at the Coweeta Hydrologic Laboratory in southwestern N.C., U.S.A., which had been ongoing since 2000 (Rosemond et al. 2008). The overall study design consisted of two headwater streams, a reference stream and a treatment stream. The treatment stream was continuously enriched with nitrogen (N) and phosphorus (P) along its entire reach (150 m). Prior to enrichment, the reference and treatment streams had similar nutrient concentrations (Reference - dissolved inorganic nitrogen (DIN): 23.2 ± 8.5 μg L^{-1}, soluble reactive phosphorus (SRP): 6.8 ± 3.0 μg L^{-1}; treatment - DIN: 29.3 ± 4.9 μg L^{-1}, SRP: 9.5 ± 2.3 μg L^{-1}). During enrichment (July 2000-June 2006), nutrient concentrations in the reference stream remained low (DIN: 31.0 ± 3.4 μg L^{-1}, SRP: 8.0 ± 1.3 μg L^{-1}), while the nutrient addition in the treatment stream produced a moderate level of enrichment (DIN: 506.2 ± 36.3 μg L^{-1}, SRP: 80.0 ± 5.6 μg L^{-1}). Further details of the irrigation system used for enrichment, as well as water sampling and analysis are presented in (Gulis and Suberkropp 2003b).

Leaf Packs

Two leaf types that differ in their rates of breakdown, red maple (*Acer rubrum* L.) and rhododendron (*Rhododendron maximum* L.), were collected in the fall and allowed to air dry for

at least two weeks. Red maple or rhododendron leaves (15g) were then placed in coarse mesh bags and added to both a reference and treatment stream on 4 December 2004. Leaf packs were periodically retrieved from the streams, rinsed, and leaf discs removed for determination of microbial activity (respiration) and fungal and bacterial biomass (below). Retrieval of replicate leaf packs (3-5 from each stream) occurred frequently in the first few weeks after deployment and less frequently later. Remaining leaf material was dried at 60ºC, weighed, and combusted at 500ºC to determine ash-free dry mass (AFDM) remaining. Mass of leaf discs removed was added back in to the total dry mass remaining. Leaf breakdown rate (k) was determined by linear regression of ln-transformed data.

Fungal biomass

Fungal biomass was estimated by measuring ergosterol content of leaf material. Ten leaf discs from each leaf pack were placed in 5 mL of methanol. Ergosterol concentration was determined by extraction and separation followed by analysis with High Pressure Liquid Chromatography (HPLC) at 282 nm (Newell et al. 1988; Suberkropp and Weyers 1996). Ergosterol concentration was converted to fungal biomass using a conversion factor of 5.5 mg ergosterol g^{-1} fungal biomass (Gessner and Chauvet 1993).

Bacterial biomass

Bacterial biomass was estimated by image analysis using epifluorescence microscopy following staining with SYBR Gold (Noble and Fuhrman 1998). Bacterial samples from FBOM (2.5 mL aliquots) were preserved in 5% buffered formalin (final concentration – 2.5%). Ten leaf discs from each leaf pack were placed in 5 mL of 2% formalin. Bacteria were removed from FBOM and leaf substrates by sonicating for 1.5 minutes using a Bransonic 150 probe sonicator, placing samples on ice every 30 seconds to prevent excessive heating of the sample (Buesing and

Gessner 2002). Samples were then centrifuged at 800x g for one minute to separate bacterial cells from particulate matter to improve image analysis. FBOM samples were diluted 1:10, and then one mL of each sample was filtered through a 0.2-µm 25 mm diameter black polycarbonate filter to capture bacteria. Filters were stained with SYBR Gold (supplied at 10,000X, final concentration 25·X; Molecular Probes, Inc.) as described in (Noble and Fuhrman 1998; Lisle and Priscu 2004). Images from 20 random fields were captured from the filter surface at 1000X magnification with an Olympus BH-2 microscope and an Olympus Qcolor 3 digital camera. For each filter, 20 microscope images were captured and analyzed using the MatLab (v 7.9) image processing toolbox. Cell counts and cell body dimensions (length and width) were used to calculate cell biovolume and, in turn, mean bacterial biomass concentration (First and Hollibaugh 2008).

Macroinvertebrates

On each sample date, leaf pack contents were rinsed through nested sieves with 1 mm and 250 µm mesh sizes. Associated invertebrates were preserved in 8% formalin stained with phloxine-B for later identification, enumeration and determination of biomass. Macroinvertebrate taxa were identified to the lowest taxonomic level possible, usually genus. Chironomidae were identified as Tanypodinae (predators) or non-Tanypodinae (non-predators). All individuals were measured to the nearest 0.5 mm in length and previously published length-mass regressions were applied to estimate AFDM (Benke et al. 1999). Functional feeding groups were assigned to each taxon according to Merritt et al. (2008). Additionally, we determined relative biomass of fungi, bacteria, shredder, and other macroinvertebrates for each stream on each sample date.

Relative contribution model

In an attempt to quantify relative roles of leaf-associated organisms responsible for the biological portion of leaf breakdown, Hieber and Gessner (2002) developed a framework that includes estimates of shredder feeding rates, growth rates and efficiencies of fungi and bacteria, and biomass of each group. We used leaf packs containing rhododendron leaves to estimate percent contribution of each group of detritivores on sample dates between which a significant mass loss occurred (d 14, 49, and 108 in the reference stream; d 7, 14, 28, 37, and 49 in the treatment stream). Because data were not available for all compartments on all sample dates, we compared a single sample date (d 14) for red maple substrates in both streams. The general form of the model is:

$$\frac{dm}{dt} = -g_s(m, B_s) - g_f(m, B_f) - g_b(m, B_b)$$

Where m is dry mass of leaf litter, t is time elapsed after litter input, B is biomass of each group, g_s is a function of shredder feeding, and g_f and g_b are functions of fungal and bacterial assimilation, respectively.

The specific components of the model were calculated for each group as follows:

Shredder feeding: $$g_s(m, B_s) = \varphi \frac{m}{m + c} B_s$$

Where φ is the maximum feeding rate (relative consumption rate) and c is a constant that is small relative to the initial leaf mass (i.e. $m/(m + c)$ accounts for the fact that feeding ceases when mass remaining approaches zero). Relative consumption was determined from studies of dominant taxa in these streams (Eggert and Wallace 2003).

Fungal assimilation: $$g_f(m, B_f) = \left(\frac{\mu_f}{\varepsilon_f} \right) B_f m$$

Where μ is gross growth rate and ε is fungal growth efficiency. Bacterial assimilation was calculated using the same formula. Growth rates and efficiencies were based on literature values (Suberkropp 1991; Weyers and Suberkropp 1996).

Data Analysis

A two-way analysis of variance (ANOVA) was used to test for main and interactive effects of nutrient enrichment and time on fungal, bacterial, and shredder biomass. In this analysis, time was treated as an independent fixed effect.

Results

Nutrient enrichment increased breakdown rates of both red maple (*Acer rubrum*) and rhododendron (*Rhododendron maximum*) by 416 % and 330%, respectively (Fig. 2.1, Table 2.1). Days to 95% mass loss was 248 in the reference stream compared to 48 in the treatment stream for red maple; for rhododendron, days to 95% mass loss was 611 in the reference stream compared to 139 in the treatment stream.

Fungal biomass peaked at an earlier date and was higher (ANOVA, $p < 0.001$) in the treatment stream, while shredder biomass peaked either at the same time or shortly after peaks in fungal biomass (Fig. 2.2, Table 2.2). Bacterial biomass was variable in the reference stream, but increased steadily in the treatment stream (d 49 biomass increased more than an order of magnitude compared to d 7, Table 2.2). On the final sample date in the treatment stream, shredders dominated macroinvertebrate biomass, while non-shredder species dominated the reference stream (Fig. 2.2). In the treatment stream, the caddisfly *Pycnopsyche* was by far the most dominant taxon in the leaf packs (Appendix A). Non-shredder taxa in the leaf packs were primarily composed of predators, such as the stoneflies *Beloneuria* and *Isoperla*, and collector-gatherers, primarily non-predator chironomids (Appendix A).

Application of the Hieber-Gessner framework to data from the reference and treatment streams indicated a change in detritivore vs. decomposer contribution over time. In both streams, the relative proportion of leaf mass loss due to shredders increased over time, while fungal contribution to mass loss peaked early and declined (Fig. 2.3). Peaks in both fungal and shredder contribution to breakdown occurred much earlier in the treatment stream. Overall, bacterial contributions to mass loss were low relative to fungi and shredders (Fig. 2.3). At similar stages of mass loss (~20%), fungi contributed as much as shredders to breakdown in the treatment stream, while shredders dominated biological contributions to breakdown in the reference stream (d 28 in the treatment stream, d 108 in the reference; Fig. 2.1 and 2.3). Compared to rhododendron, model estimates for red maple on d 14 were low for all three detritivore groups in both the reference and treatment streams (Fig. 2.4). Mass loss on d 14 in the reference stream was 7% for rhododendron and 15% for red maple compared to 25% and 60% in the treatment for rhododendron and red maple, respectively.

Discussion

Experimental nutrient addition resulted in leaf litter breakdown rates that were 4-5X higher than under reference conditions. Increased rates were associated with increased biomass of both microorganisms and macroinvertebrates. In the treatment stream, fungal biomass peaked earlier in the decay sequence than in the reference stream. By d 14 of incubation, shredder biomass on rhododendron leaf litter in the treatment stream was as high as values in the reference stream that did not occur until d 49. But, since mass loss was much slower in the reference stream, at similar stages of mass loss (~20%; d 28 and 108 in the treatment and reference streams, respectively), fungi contributed as much as shredders to breakdown in the treatment stream, while shredders dominated biological contributions to breakdown in the reference stream. For a

similar incubation time, macroinvertebrate contributions to leaf mass loss were greater than losses due to microbes, and processing attributed to biotic components was greater in the treatment vs. the reference stream.

Acceleration of carbon loss has been found in response to many types of land use changes and other kinds of global change (Luo and Weng 2011; Poeplau et al. 2011). Increases in breakdown rates in the treatment stream relative to the reference stream were as high or higher in year five of enrichment (this study) than years one and two of enrichment (Greenwood et al. 2007). Other studies have also shown increases in breakdown with addition of nutrients (Meyer and Johnson 1983; Ferreira et al. 2006). In some cases, streams affected by multiple stressors may exhibit decreases in leaf litter breakdown despite nutrient enrichment (Pascoal et al. 2005). As streams are affected by various inputs, nutrient effects on consumers and organic matter resources become more complex and difficult to predict (Baldy et al. 2007; Imberger et al. 2008).

Effects of shredders on leaf mass loss remained important with nutrient enrichment relative to microbial decomposers and accounted for approximately 25% of mass loss on d 108 on rhododendron. By d 49 in the treatment stream, the caddisfly *Pycnopsyche* was by far the most dominant macroinvertebrate in the leaf packs. In similar streams, reductions in shredders have been associated with decreases in leaf breakdown rates, suggesting their importance in organic matter processing (Wallace et al. 1996). Other studies, however, have suggested that microbial biomass, particularly fungi, drive increased breakdown rates in impacted streams where shredders are scarce (Pascoal et al. 2005; Imberger et al. 2008). Fungi typically account for a greater proportion of the total microbial production associated with leaf litter than bacteria (Pascoal and Cassio 2004). Fungi also tend to dominate the early stages of breakdown (Gessner

and Chauvet 1994), thus conditioning leaves and providing increased nutrients to shredders (Graca 2001).

Although this increase in mass loss was likely facilitated by increased microbial conditioning, and shredders also played a role in this acceleration, biological contributions to mass loss only accounted for a maximum of 35%. Our estimates of relative contribution using the Hieber and Gessner (2002) framework show a much lower total value from the biotic compartment than their estimates from the Steina River in Germany. At d 55 (74 and 92% mass loss for willow and alder, respectively), their sum of estimated contributions from all three compartments of the model was 78 and 91% for willow and alder leaf packs, respectively. Both willow and alder are labile species with relatively high breakdown rates (Webster and Benfield 1986; Hieber and Gessner 2002). Although we only have a single, early date for comparison with the more labile red maple (d 14, 37 and 49% mass loss for the reference and treatment streams, respectively), total contribution of detritivores and decomposers to leaf litter breakdown was low, suggesting that abiotic factors contributed a great deal to breakdown in both labile and recalcitrant species.

In our high gradient, mountain streams, it is not surprising that we observed much greater leaf mass loss due to physical abrasion from high flow than in rivers like the low gradient Steina (Hieber and Gessner 2002). Differences in biotic contribution to breakdown between this study and the Hieber-Gessner study should be expected given differences, not only in structural characteristics of leaf species, but also in physical characteristics of the respective study streams. Increased breakdown rates have been associated with high flows during the wet season in Hong Kong (Niu and Dudgeon 2011). Higher stormwater runoff has also been suggested as a cause of increased breakdown rates in urban streams (Paul et al. 2006). Our data do suggest that, in the

presence of elevated nutrients, biological response likely exacerbates the effects of abiotic factors such as stream flow. Earlier and higher peaks in fungal biomass on rhododendron caused by increased nutrients in the treatment stream are not only likely to increase breakdown rates directly, but microbial conditioning also softens leaf tissue, resulting in further increases in mass loss due to abiotic factors, particularly flow.

Although the application of the framework elucidated changes in relative contribution of fungi and shredders with nutrient enrichment, there were limitations. For simplicity, microbial growth rates and efficiencies and shredder consumption rates were held constant over time and between streams. Realistically, these parameters will change over time and with changes in available nutrients. Other studies in these streams have shown an increase in fungal growth rates in the treatment stream, and these growth rates change with incubation time (Gulis et al. 2008). Adjusting this growth rate to the maximum value measured in the treatment stream increases the relative contribution of fungi by 75% in the treatment stream relative to values calculated using published growth rates consistent with those in the reference stream. The value assigned for shredder consumption rate was an average of previously determined consumption rates of the three most dominant shredders in our study streams (Eggert and Wallace 2003). These rates vary among species, so will also change as relative abundances change over time. Incorporating interactions among detritivores and decomposers could also strengthen this model. Changes in how these organisms assimilate resources and respond to one another are likely important in determining effects on ecosystem processes.

Increased breakdown rates, particularly of those recalcitrant species such as rhododendron that persist in streams for well over a year in low nutrient conditions, are likely to result in localized increases in carbon loss via respiration and downstream transport of FBOM

(Benstead et al. 2009). A reduction in persistence of leaf litter in streams as a result of nutrient enrichment may have important implications for consumers. In our study streams, leaf litter standing crop in the treatment stream approached zero each year of enrichment several months earlier than the reference stream (Suberkropp et al. 2010). Based on our results, more labile coarse particulate organic matter, like red maple that would be available at least through spring, is likely to disappear by early winter under conditions of chronic enrichment. Likewise, recalcitrant resources like rhododendron, which are available to consumers for well over a year under ambient conditions, are likely to vanish prior to spring.

Application of this framework to a nutrient-enriched stream suggests a mechanism to explain how changes in N and P alter fates of carbon in this and other studies (Mack et al. 2004, Benstead et al. 2009). Shifts toward increasing fungal and shredder contribution to leaf litter breakdown with enrichment may increase downstream export through increased fragmentation of leaf material, production of fecal material by macroinvertebrates, and microbial conditioning. Nutrient enrichment has the potential to greatly alter the quantities of carbon processed by inland waters (Cole et al. 2007). Because headwater streams are the origin of river networks and account for a majority of estimated stream miles, understanding how these ecosystems respond to nutrient enrichment is critical in developing proper management strategies (Leopold et al. 1964, Meyer and Wallace 2001).

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Table 2.1. Breakdown coefficients (k) and days to 95% loss for red maple and rhododendron leaf packs during year 5 of nutrient enrichment in the reference (Ref) and treatment (NP) streams at the Coweeta Hydrological Laboratory. Breakdown coefficients are calculated as the negative slope of ln percent leaf AFDM remaining versus days in stream.

	k		Days to 95% loss	
	Ref	**NP**	**Ref**	**NP**
Red Maple	0.012	0.062	248	48
Rhododendron	0.005	0.022	611	139

Table 2.2. Average values of fungal, bacterial, and shredder biomass (mg C g $AFDM^{-1}$) on rhododendron substrates at individual sample dates from December 2004-March 2005. Asterisks indicate significant differences between the treatment and reference stream on a given date based on Tukey's multiple comparison tests ($p < 0.05$). ND = no data.

Days in Stream	7	14	28	37	49	108
Reference						
Fungal biomass	0.69	1.00	1.87	3.64	3.84	0.92
Bacterial biomass	0.04	0.01	0.01	0.01	0.04	0.02
Shredder biomass	0.30	0.83	0.52	0.84	1.84	1.18
Mass loss (%)	2.3	7.0	6.2	11.2	11.7	18.7
Treatment						
Fungal biomass	0.76	1.90	15.37*	20.53*	16.17*	ND
Bacterial biomass	0.02	0.02	0.04	0.06	0.58*	ND
Shredder biomass	0.41	1.93	4.35*	9.08*	49.78*	ND
Mass loss (%)	3.1	7.5	20.3	37.6	56.4	ND

Fig. 2.1. Ash-free dry mass (AFDM) remaining of a) red maple and b) rhododendron in leaf packs placed in the reference and treatment streams at Coweeta Hydrological Laboratory. Ref = reference stream; NP = treatment stream.

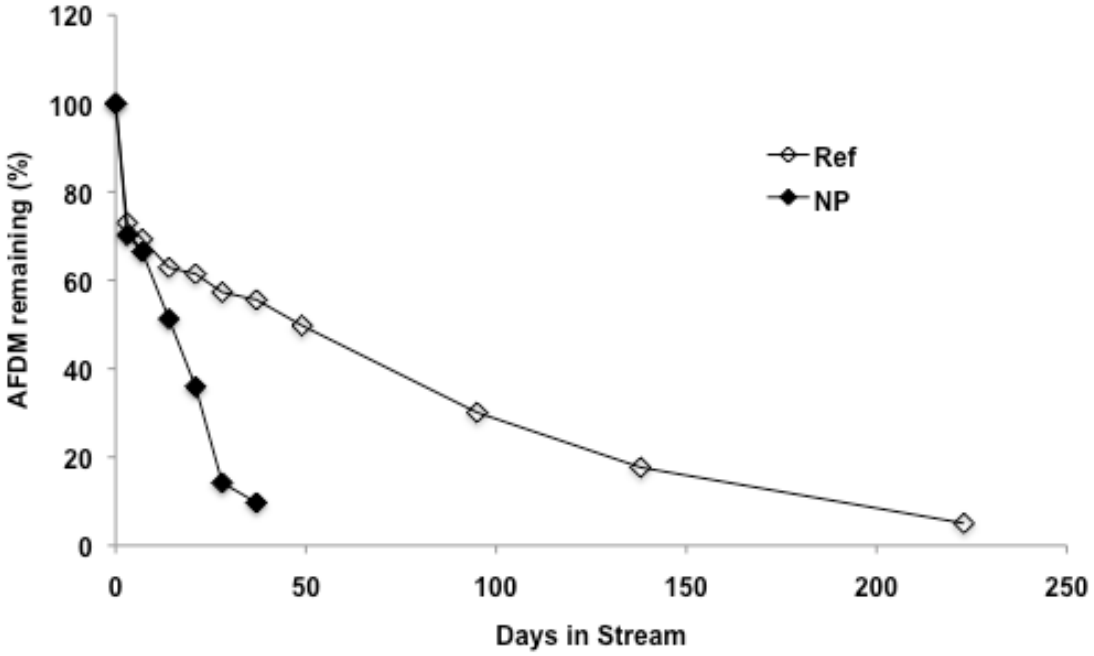
Fig. 2.2. Relative biomass (%) of bacteria, fungi, shredders, and other macroinvertebrates in rhododendron leaf packs in the a) reference stream through day 108 and b) treatment stream through day 49.

Fig. 2.3. Estimated relative contribution (%) to rhododendron leaf litter mass loss for bacteria, fungi, and shredders in the a) reference and b) treatment streams.

Fig. 2.4. Estimated relative contribution (%) to maple leaf litter mass loss for bacteria, fungi, and shredders after 14 days in the stream in the reference (Ref) and treatment (NP) streams.

Fig. 2.1.

a)


AFDM remaining (%)
120
100
80
60
40
20
0
0
50
100
150
200
250
Days in Stream
Ref
NP

b)

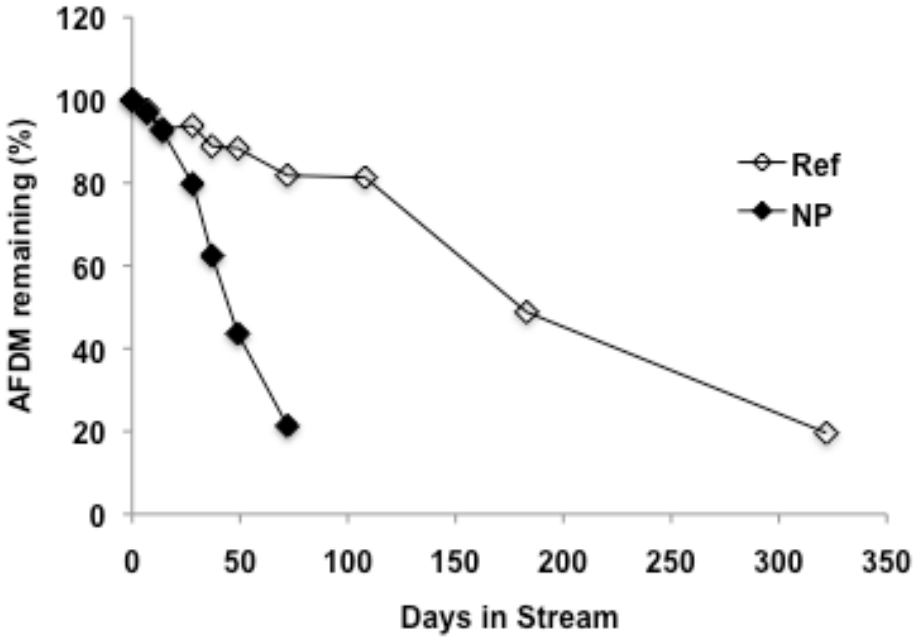
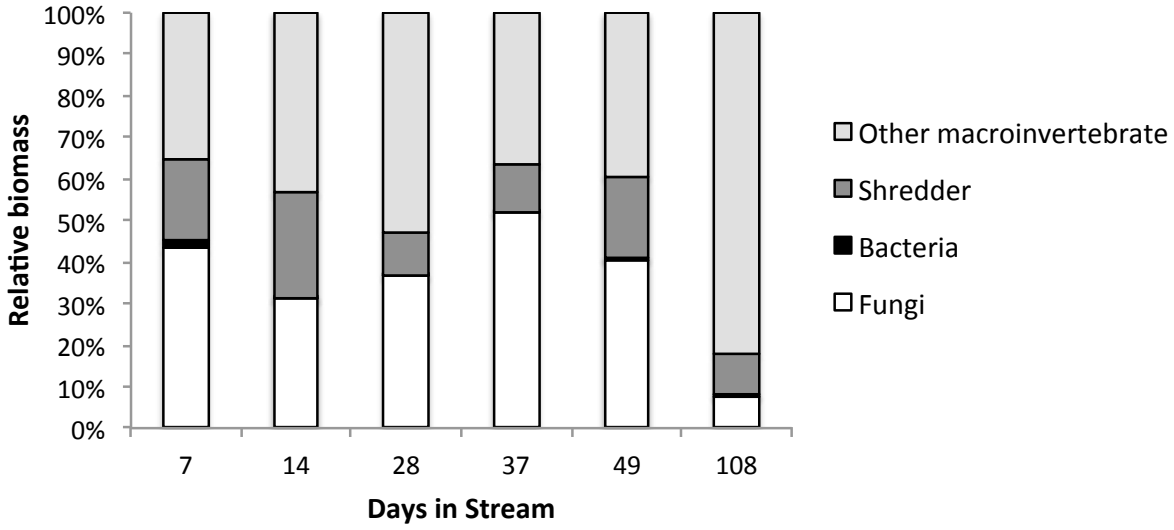

AFDM remaining (%)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Days in Stream
Ref
NP

Fig. 2.2.

a)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative biomass
7
14
28
37
49
108
Days in Stream
Other macroinvertebrate
Shredder
Bacteria
Fungi

b)

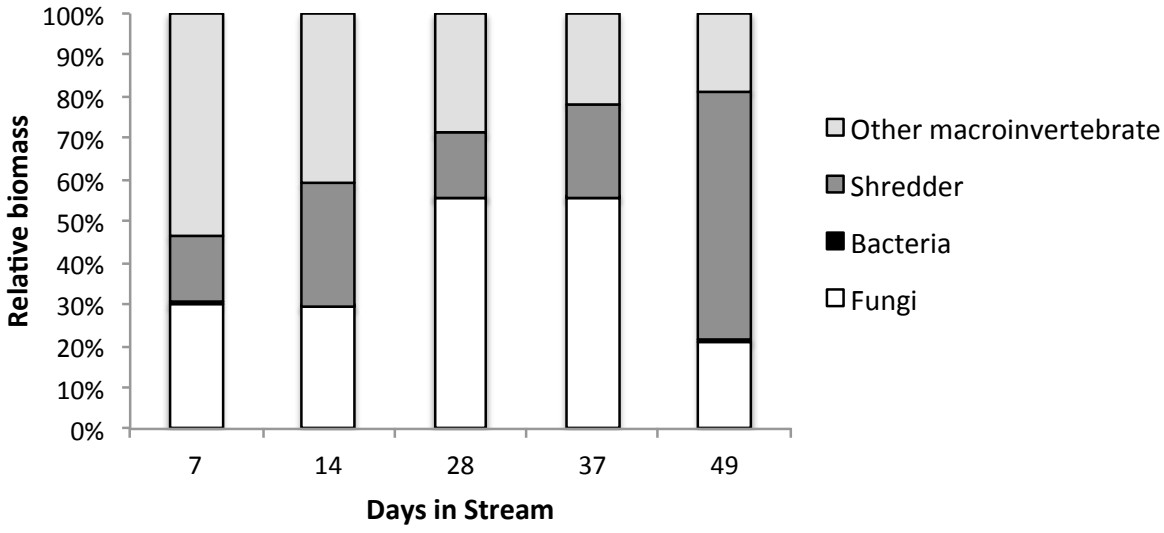
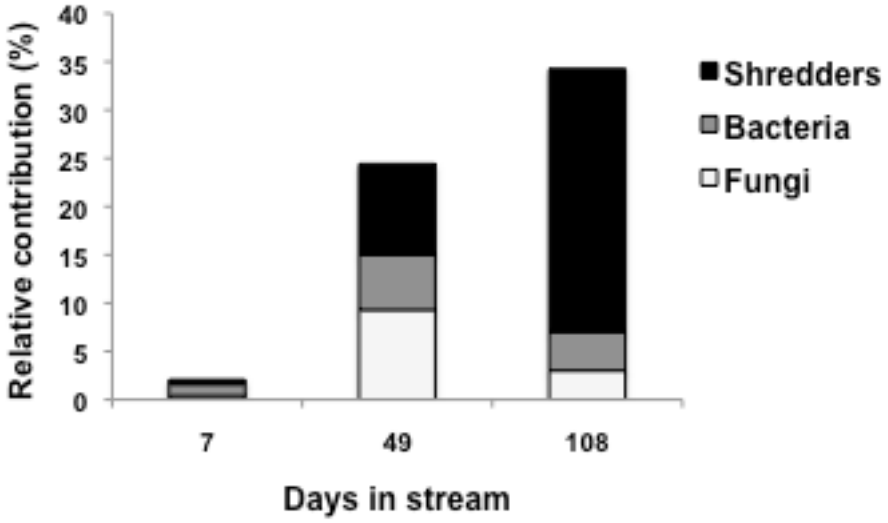

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative biomass
7
14
28
37
49
Days in Stream
Other macroinvertebrate
Shredder
Bacteria
Fungi

Fig. 2.3.

a)


Relative contribution (%)
40
35
30
25
20
15
10
5
0
7
49
108
Days in stream
Shredders
Bacteria
Fungi

b)

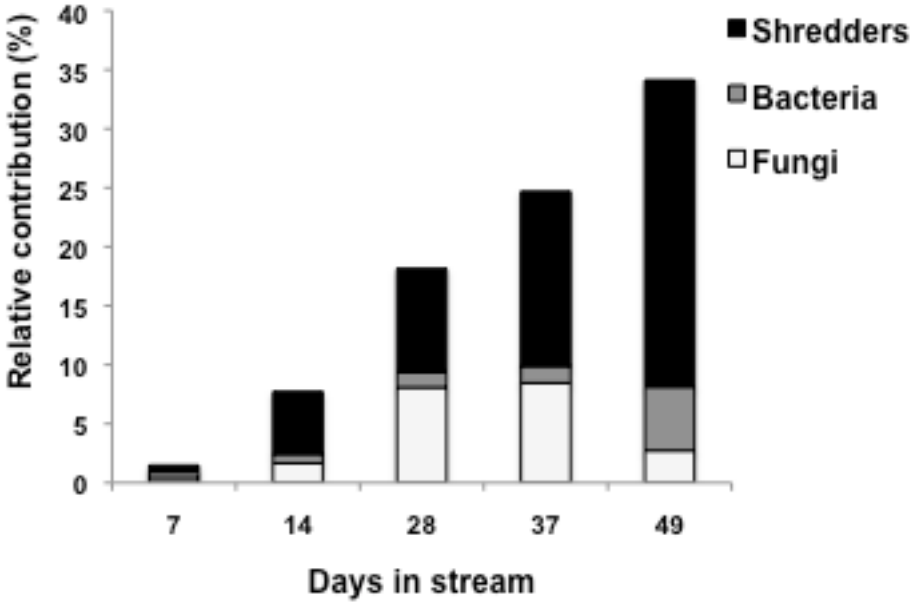
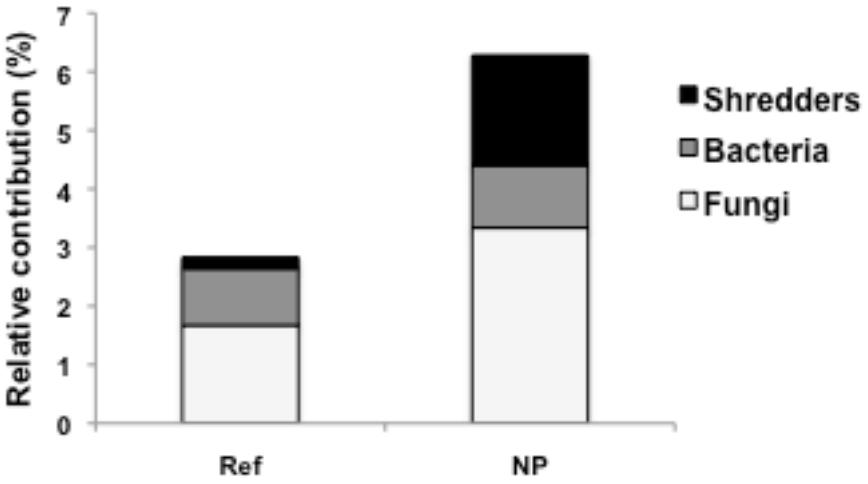

Relative contribution (%)
40
35
30
25
20
15
10
5
0
7
14
28
37
49
Days in stream
Shredders
Bacteria
Fungi

Fig. 2.4.


Relative contribution (%)
0
1
2
3
4
5
6
7
Ref
NP
Shredders
Bacteria
Fungi

CHAPTER 3

DIFFERENTIAL EFFECTS OF NUTRIENT ENRICHMENT ON FINE AND COARSE ORGANIC MATTER RESOURCES IN A HETEROTROPHIC STREAM: IMPLICATIONS FOR NUTRIENT LIMITATION OF CONSUMERS[2]

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Abstract

Nutrient enrichment has been shown to affect heterotrophic microorganisms (bacteria and fungi) associated with dead organic matter, but little is known about how biota associated with diverse size fractions of organic matter (detritus) respond to nutrients. Fine (<1 mm) vs. coarse (>1 mm) fractions of detritus are dominated by different groups of microorganisms (bacteria and fungi, respectively) and are utilized by different groups of consumers. Here, we quantified fungal and bacterial biomass, microbial respiration, and detrital nutrient content on fine benthic organic matter (FBOM) and coarse particulate organic matter (CPOM), two types of leaf litter, in response to an experimental whole-stream nutrient enrichment. We used the carbon:nutrient ratios measured in this study to examine the implications of shifting nutrient ratios on macroinvertebrate consumer biomass. Nutrient enrichment increased fungal biomass, microbial respiration and detrital nutrient content on both maple and rhododendron substrates. Bacterial biomass on rhododendron also increased. However, only bacterial biomass and microbial respiration increased on FBOM. Greater changes were observed in detrital carbon:phosphorus (C:P) than carbon:nitrogen (C:N) and maximum changes in nutrient content occurred on the leaf type with the highest initial carbon:nutrient ratio. Both bacterial and fungal biomass were associated with changes in CPOM C:N and C:P. To predict how these changes in detrital resources may differentially affect consumers, we determined threshold elemental ratios for two important groups of detritivores. Our results indicated that enrichment may reduce the severity of P limitation for shredders, which feed on CPOM, but may exacerbate C limitation for collector-gatherers, which feed on FBOM. This analysis highlights potential mechanisms for predicted shifts in FBOM vs. CPOM food webs and carbon flow that may result from nutrient enrichment of detritus-based systems.

Introduction

Increased nutrient mobilization and associated increased bioavailability of nitrogen (N) and phosphorus (P) is a global problem in freshwater ecosystems (Smith and Schindler 2009). The effects of nutrient enrichment on systems dominated by heterotrophic pathways (defined as those where carbon (C) obtained for growth and metabolism is derived from detrital carbon, rather than directly from autotrophic carbon) have been studied little compared to autotrophic food webs. Predictions of nutrient enrichment effects on heterotrophic state include increased respiration of carbon inputs driven by organisms from heterotrophic microbes to higher order consumers (Dodds 2007). However, very little is known about how different types of organic carbon resources (small vs. large size fractions) or different groups of heterotrophic microbes respond to nutrient enrichment.

In stream ecosystems, particulate organic carbon resources can be divided into two distinct functional types: coarse and fine particles. Coarse detritus in the form of leaves and wood provides the majority of energy and nutrients in many forested headwater stream food webs, but fine benthic organic matter is also a significant resource for consumers and dominates the basis of consumer production in larger rivers (Wotton 1994; Wallace et al. 1997; Rosi-Marshall and Wallace 2002). In general, detrital nutrient content increases with reductions in particle size (Stelzer et al. 2003). Microbial assemblages, composed of fungi and bacteria, are important primary consumers of these resources. The relative biomass of bacteria and fungi differs on detrital size fractions; fungi dominate on coarse particulate organic matter (CPOM) (i.e. wood and leaves), and bacteria dominate on fine benthic organic matter (FBOM) (Findlay et al. 2002). Heterotrophic microbes attached to organic matter are potentially important drivers in facilitating changes in particulate organic matter quality via increases in nutrient content. Fungi and bacteria

differ in elemental content and have lower carbon to nutrient ratios than the particulate organic matter that they colonize (Stelzer et al. 2003). Their association with low quality particulate matter therefore increases its nutrient content, reduces carbon to nutrient ratios, and increases its nutritional value for detritivores (Arsuffi and Suberkropp 1985).

Because detritus is a poor quality resource even when it is colonized by microbial biomass under ambient nutrient concentrations, nutrient enrichment can cause dramatic changes in detrital quality (Robinson and Gessner 2000; Grattan and Suberkropp 2001; Pascoal et al. 2005). However, this quality response may differ for small- and large-sized detrital particles as a result of fungal vs. bacterial dominance or the unique chemical characteristics of substrates (i.e. differences in initial carbon:nutrient ratios). For instance, increased fungal response due to enrichment should lead to greater changes in CPOM, but may have less impact on FBOM quality due to its increased dominance on CPOM vs. FBOM. Conversely, increased bacterial response would be predicted to lead to greater changes in FBOM than CPOM because of its greater dominance on finer particles (Findlay et al. 2002). Previous studies have shown that the response to nutrient enrichment was greater for substrates that were higher vs. lower in C:N, as is the case for wood vs. leaf litter (Stelzer et al. 2003; Gulis et al. 2004) as well as leaf litter of different species that differ in C:nutrient content (Greenwood et al. 2007). In addition, CPOM has been shown to have a greater magnitude change in C:nutrient ratios with enrichment than FBOM, presumably because of high ambient C:nutrient ratios of CPOM (Cross et al. 2005). While it is assumed that microbial response drives these trends, the relationship between fungal and bacterial biomass and substrate quality under enriched conditions has not been determined. It is not clear if the greater response of higher C:nutrient substrates to enrichment is due primarily to increases in fungal biomass as has been postulated.

Because shredders are the primary consumers of CPOM, and collector-gatherer-gatherers feed primarily on FBOM, differential effects of nutrient enrichment on CPOM vs. FBOM could have contrasting effects on limiting factors of these two functional food groups. These changes may lead to divergent cascading effects on energy flow through these two food web pathways. If nutrient enrichment decreases C:nutrient ratios on CPOM to a greater extent than on FBOM, this could facilitate energy flows through shredder pathways rather than collector-gatherer pathways. In order to assess how nutrient enrichment on CBOM vs. FBOM may differentially affect these two groups of consumers, we calculated threshold elemental ratios (TERs). TERs estimate the resource carbon:nutrient ratio at which limitation of consumer growth shifts from one element to another and are therefore useful for estimating elemental imbalances between consumers and their food (Frost et al. 2006). By increasing food quality of detrital food resources (lower C:N and C:P), nutrient enrichment may alter consumer production through changes in consumption and growth rates with subsequent effects on carbon flow and retention. Estimates of macroinvertebrate abundance and biomass in these streams have shown a disproportionately greater response to enrichment by large-bodied primary consumers, in particular the shredder *Pycnopsyche* (Davis et al. 2010).

In the present study, we quantified and compared bacterial and fungal biomass and microbial activity (respiration) on CPOM and FBOM and determined changes in detrital nutrient content. As a way to assess implications of changes in detrital nutrient content on consumers, we also determined consumer (TERs) and compared changes in detrital nutrient content to estimated consumer requirements. We predicted that nutrient enrichment would have differential effects on fine (< 1 mm) and coarse (> 1 mm) organic matter fractions. Specifically, we predicted that: (1) nutrient enrichment would stimulate fungal and bacterial biomass, respiration rates, and

associated nutrient content on both substrates; (2) changes in nutrient content with enrichment would be greater on CPOM due to initially higher C:N and C:P ratios on those substrates relative to FBOM; and (3) nutrient content would change as a function of fungi on CPOM and bacteria on FBOM because of their relative dominance on the substrates. Additionally, we quantified change in fungal biomass, bacterial biomass, respiration rates and nutrient content on fine vs. coarse detritus (for both leaf types). Our experimental enrichment provided both N and P above concentrations considered to be growth limiting to heterotrophic microbes (Rosemond et al. 2008). Thus, we tested for relative changes in substrate C:N vs. C:P that would indicate differential uptake of N vs. P by detritus-associated microbes.

Methods

Nutrient enrichment

This study was conducted within the framework of a long-term nutrient enrichment conducted in a paired watershed study at the Coweeta Hydrologic Laboratory in southwestern North Carolina, U.S.A., which was continuously enriched from 2000-2006 (Rosemond et al. 2008). Data presented here are from samples were collected during years five and six of enrichment (December 2004-June 2006). The overall study design consisted of two first order headwater streams, a reference stream and a treatment stream. The treatment stream was continuously enriched with nitrogen (N) and phosphorus (P) along its entire reach (150 m). Prior to enrichment, the reference and treatment streams had similar nutrient concentrations (Reference - dissolved inorganic nitrogen (DIN): 23.2 ± 8.5 μg L^{-1}, soluble reactive phosphorus (SRP): 6.8 ± 3.0 μg L^{-1}; treatment - DIN: 29.3 ± 4.9 μg L^{-1}, SRP: 9.5 ± 2.3 μg L^{-1}). During enrichment (July 2000-June 2006), nutrient concentrations in the reference stream remained low (DIN: 31.0 ± 3.4 μg L^{-1}, SRP: 8.0 ± 1.3 μg L^{-1}), while the nutrient addition in the treatment

stream produced a moderate level of enrichment (DIN: 506.2 ± 36.3 μg L^{-1}, SRP: 80.0 ± 5.6 μg L^{-1}). Further details of the irrigation system used for enrichment, as well as water sampling and analysis are described elsewhere (Gulis and Suberkropp 2003).

Organic matter sample collection

For our analysis of effects of nutrients on CPOM, we deployed two leaf litter types, red maple (*Acer rubrum*) and rhododendron (*Rhododendron maximum*), that differ in their rates of breakdown and initial carbon:nutrient ratios. Recently senesced leaves were collected in the fall and allowed to air dry for at least two weeks. Red maple or rhododendron leaves (15g) were placed in coarse mesh bags and deployed in each stream on 14 December 2004. Leaf packs were periodically retrieved from the streams, rinsed, and leaf discs removed for determination of microbial activity (respiration) and fungal and bacterial biomass. Retrieval of replicate leaf packs (n = 3) occurred frequently in the first few weeks after deployment and less frequently later. Remaining leaf material was dried at 60ºC, weighed, and combusted at 500ºC to determine ash-free dry mass (AFDM) remaining.

For our analysis of nutrient effects on FBOM, it was not feasible to deploy a previously uncolonized substrate, so we based our analysis on collections of standing material in reference and treatment streams over multiple dates (27 June 2005-30 June 2006). Sampling in this manner ensured that FBOM sampled had been exposed to enrichment for an extended period of time. Between three and five replicate grab samples of FBOM were collected at each sampling time. A 100 mL slurry was removed from each sample for measurement of respiration in the field, and the remaining volume of sample was placed in a cooler, returned to the lab, and preserved for fungal and bacterial biomass determinations (described below).

Microbial respiration

CPOM-associated microbial respiration, measured as oxygen uptake (mg O_2 g leaf $AFDM^{-1}$ hr^{-1}), was determined by placing 10 leaf discs from each leaf pack in 30 mL of stream water in respiration chambers to measure decrease in oxygen concentration over time. Leaf discs were then dried at 60°C, weighed, and combusted at 500ºC to determine AFDM. Respiration for each leaf type was measured on each retrieval date until adequate leaf material was no longer available.

FBOM-associated respiration was measured by incubating samples for two hours in 150 mL BOD bottles to measure decrease in oxygen concentration over time. Samples (100 mL) were placed in BOD bottles and then filled to the top with filtered stream water. Initial and final measurements of oxygen (mg L^{-1}) were recorded with a YSI 5100 dissolved oxygen meter and YSI 5010 BOD probe to determine mg O_2 consumed g $AFDM^{-1}$ hr^{-1}. Following completion of respiration measurements, each 100 mL sample was filtered through a 0.7 micron pore size glass fiber filter, dried at 60ºC, weighed, and combusted at 500ºC to determine ash-free dry mass (AFDM).

Microbial biomass

Fungal biomass was estimated by measuring ergosterol content of leaf material and FBOM. Ten leaf discs from each leaf pack were placed in 5 mL of methanol. Fungal samples from FBOM (25 mL aliquots) were filtered through a 0.7 m glass fiber filter and placed in 5 mL of methanol. Ergosterol concentration was determined by extraction and separation followed by analysis with High Pressure Liquid Chromatography (HPLC) at 282 nm (Newell et al. 1988; Suberkropp and Weyers 1996). Ergosterol concentration was converted to fungal biomass based on standards.

Bacterial biomass was estimated by image analysis using epifluorescence microscopy following staining with SYBR Gold (Noble and Fuhrman 1998). Bacterial samples from FBOM (2.5 mL aliquots) were preserved in 5% buffered formalin (final concentration – 2.5%). Ten leaf discs from each leaf pack were placed in 5 mL of 2% formalin. Bacteria were removed from FBOM and leaf substrates by sonicating for 1.5 minutes using a Bransonic 150 probe sonicator, placing samples on ice every 30 seconds to prevent excessive heating of the sample (Buesing and Gessner 2002). Samples were then centrifuged at 800xg for one minute to separate bacterial cells from particulate matter to improve image analysis. FBOM samples were diluted 1:10, then one mL of each samples was filtered through a 0.2 μm 25 mm diameter black polycarbonate filter to capture bacteria. Filters were stained with SYBR Gold (supplied at 10,000X, final concentration 25·X; Molecular Probes, Inc.) as described in (Noble and Fuhrman 1998; Lisle and Priscu 2004). Images from twenty random fields were captured from the filter surface at 1000X magnification with an Olympus BH-2 microscope and an Olympus Qcolor 3 digital camera. For each filter, 20 microscope images were captured and analyzed using the MatLab (v 7.9) image processing toolbox. Cell counts and cell body dimensions (length and width) were used to calculate cell biovolume and, in turn, mean bacterial biomass concentration (First and Hollibaugh 2008).

Carbon, nitrogen, and phosphorus content

Leaves from leaf packs and 25 mL subsamples of FBOM were dried at 60ºC and ground in a ball mill prior to analysis for C, N, and P content. Ground material was then weighed and analyzed for C and N content with a Carlo Erba NA 1500 CHN analyzer. Phosphorus content was analyzed spectrophotometrically after acid digestion (APHA 1998). Nutrient ratios were expressed on a molar basis.

Statistical Analyses

Because only one reference and one enriched stream were used in this study, analyses were not strictly replicated, violating assumptions of inferential statistics (Hurlbert 1984). However, the ecosystem-level scale of the experiment provided results consistent with system-level conditions, and the paired watershed approach has been utilized as an effective way to examine ecosystem processes through experimental manipulation (Carpenter 1989). Regardless, nutrient effects based on replicate and spatially distributed samples within streams should be interpreted with caution.

A two-way analysis of variance (ANOVA) was used to test for main and interactive effects of nutrient enrichment and time on fungal biomass, bacterial biomass, microbial respiration, C:N, and C:P. In this analysis, time was treated as an independent fixed effect. Effects of nutrient enrichment, leaf species, and microbial biomass on substrate nutrient content were analyzed with two-way analysis of covariance (ANCOVA). Nutrient enrichment and leaf species were treated as fixed effects, and measures of microbial biomass were used as cofactors. Linear regressions were used to compare the response between treatments in cases where nutrient x leaf species were significant. We additionally ran one-way ANCOVAs for CPOM and FBOM resources to test whether changes in C:P relative to C:N occurred due to nutrient enrichment. Data were transformed using the natural logarithm to meet assumptions of normality and homoscedasticity. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC 27513, USA).

Estimation of Threshold Elemental Ratios

We determined threshold elemental ratios (TERs) to evaluate changes in detrital resources relative to nutritional requirements of consumers. TERs were estimated using the following published formula from (Frost et al. 2006).

$$TER_{C:nutrient} = (A_{nut}/GGE_C)(Q_C/Q_{nut})$$

where A is the assimilation efficiency of the nutrient N or P, GGE_C is the gross growth efficiency of the consumer based on C ingested, Q_C/Q_{nut} is the proportion of C:nutrient in consumer dry mass. Assimilation efficiency for N and P was set at 0.8 and gross growth efficiency for C was set at 0.2. These values were determined by Frost et al. (2006) from literature sources. Shredder and collector-gatherer body C:P and C:N used in determinations of TERs were taken from data collected from these same study streams published in Cross et al (2003).

Results

Nutrient enrichment effects on microbial biomass and activity

Nutrient enrichment resulted in higher fungal and bacterial biomass and microbial respiration on CPOM of both litter types (red maple and rhododendron), but only affected microbial respiration and bacterial biomass on FBOM (Table 3.1, Appendix B). Percentage changes in fungal and bacterial biomass and respiration were greatest for rhododendron substrates and least for FBOM (Table 3.2). Significant nutrient x date interactions on CPOM substrates were due to initially similar values of response variables during early colonization that diverged over time (Appendix B). Significant date effects (evident by using ANOVAs) were also expected due to microbial colonization on CPOM substrates and possible seasonal variation in response variables associated with FBOM.

Nutrient enrichment effects on substrate microbial content

Nutrient enrichment resulted in reduced C:N and C:P on both CPOM litter types (red maple and rhododendron), but no effect of nutrients was observed on C:N and C:P of FBOM (Tables 3.1 and 3.2, Appendix B). Percentage change in nutrient content was greater in C:P than C:N. Averaged across all samples, percentage change was greater for red maple than rhododendron, but maximum changes in C:P and C:N were greater for rhododendron than red maple (Table 3.2).

The role of microbes in driving nutrient content

To follow up on the significant effects of nutrient enrichment on CPOM nutrient content, we conducted ANCOVAs to identify and quantify the roles of microbial colonization and nutrient enrichment in driving variation in detrital nutrient content. There was a significant negative relationship between fungal biomass and C:N on CPOM substrates, and this effect did not differ with nutrient enrichment or leaf species. Specifically, neither nutrient enrichment nor species identification changed the nature of the relationship between fungal biomass and C:N (Table 3.3, Fig. 3.1a). For every one percent increase in fungal biomass, there was a 0.23% reduction in detrital C:N (Fig. 3.1a). The same trend was seen between fungal biomass and C:P, but with a significant interaction between nutrient addition and leaf species (Table 3.3). Individual regression analyses indicated a significant negative relationship between fungal biomass and C:P only on nutrient-enriched red maple and rhododendron samples, and these regressions indicated that there was 0.19% reduction in red maple C:P and a 0.25% reduction in rhododendron C:P for each one percent increase in fungal biomass (Table 3.4, Fig. 3.1b). Bacterial biomass was also associated with changes in C:N and C:P of CPOM, which did not differ due to leaf species or nutrient enrichment (Table 3.3, Fig. 3.2). For each one percent

increase in bacterial biomass, there was a 0.22% reduction in C:N and a 0.34% reduction in C:P on CPOM substrates (Table 3.4, Fig. 3.2).

Relationship between C:N and C:P

We determined whether the relationships between C:N and C:P on detrital substrates differed in the nutrient enriched vs. reference stream. On CPOM, we found a significant positive relationship between C:N and C:P and a significant difference between the reference and treatment streams, whereby CPOM was lower in C:P for a given C:N in the nutrient enriched stream over the range of values observed ($p < 0.001$, Fig. 3.3a). On FBOM, there was also a significant positive relationship between C:N and C:P, and nutrient-enriched samples had significantly lower C:P than the reference stream for a given value of C:N ($p < 0.001$, Fig. 3.3b).

Threshold Elemental Ratios

Estimation of TERs for C:N and C:P suggested that collector-gatherers were more C vs. N or P limited, while shredders were more N or P vs. C limited under reference conditions (Table 3.5). Comparisons of detrital nutrient content (FBOM for collector-gatherers, CPOM for shredders) to TERs under enriched conditions indicated that enrichment may reduce the severity of P limitation for shredders but may exacerbate C limitation for collector-gatherers.

Discussion

As predicted, we observed differential effects of nutrient enrichment on CPOM vs. FBOM, whereby response of CPOM (for both maple and rhododendron) was primarily positive and well above the response of FBOM that was unexpectedly low. On both maple and rhododendron, nutrient enrichment increased bacterial and fungal biomass and microbial respiration and decreased C:N and C:P, suggesting a general increase in resource quality.

Conversely, only bacterial biomass and microbial respiration responded positively to nutrient enrichment on FBOM.

Estimates of TERs highlight the potential for shifts in FBOM vs. CPOM food web pathways due to differential changes in food resources utilized by different functional groups. These effects may also interact with observed decreases in C quantity that occur with long-term enrichment. While some functional groups, in this case shredders, may experience increases as a result of less nutrient limitation, others, here collector-gatherers, may experience declines as a result of C limitation. These results suggest a potential mechanism to explain observed increases in some large-bodied primary consumers, such as the shredder *Pycnopsyche,* but a lack of response of smaller-bodied primary consumers under enriched conditions (Davis et al. 2010). Increased C-limitation with enrichment may also explain why declines in chironomids were observed with enrichment over time after an initial increase in the first two years of enrichment (Davis et al. 2010, In these small headwater streams, macroinvertebrate consumers have been shown to play an important role in fine particulate organic matter (FPOM) export (Wallace et al. 1991). Increased mobilization of carbon due to nutrient enrichment is likely to exacerbate the effects of these consumer interactions on downstream ecosystems (Benstead et al. 2009).

Increases in microbial respiration in the nutrient-enriched stream suggest increases in growth and metabolism. Although microbial activity increased for FBOM, this increase may not have been reflected in fungal or bacterial biomass due to loss of carbon through respiration rather than accumulation as biomass. Increased respiration has been reported in river sediments downstream of a wastewater treatment plant amended with ammonium (Ingendahl et al. 2002) as well as sediments in a South Carolina salt marsh amended with N and P (Morris and Bradley

1999). The lack of FBOM response for most variables may also be related to particle size and low initial C:nutrient ratios.

Another potential explanation for the lack of nutrient effect on FBOM substrates is the variable nature of fine particles. Variability in its source and age relative to CPOM could increase variation in response to nutrients. Unlike CPOM (in this case, leaves), FBOM consists of material from multiple sources, including erosional inputs from allochthonous sources, adsorption of dissolved organic matter, fecal material, and breakdown of CPOM, that has often been significantly altered through microbial breakdown and macroinvertebrate consumption (Ward et al. 1994). Additionally, the age of FBOM is more varied than leaf litter, given that most leaf litter enters these streams as a pulse in the fall. In many cases, FBOM may have been in the stream for a long period of time unlike the limited, although somewhat variable depending on species, amount of time that leaves remain in the system. Although grab samples were collected for FBOM, the ability to use leaf packs of known age provided more specific data as breakdown progressed in the two structurally distinct species that we selected.

Nutrient enrichment changed the relationship between fungal biomass and C:P, and this was more pronounced for more recalcitrant substrates with high initial carbon:nutrient ratios (i.e. rhododendron). Fungi presumably were better able to exploit the presence of increased water column nutrients than bacteria (Suberkropp and Chauvet 1995). A generally greater response to enrichment of rhododendron relative to red maple was likely a result of both structural and chemical differences. Rhododendron has a characteristic waxy cuticle, and freshly abscised leaves have very high C:N and C:P. Rhododendron is also high in lignin and other structural compounds characteristic of recalcitrant species. Previous studies have also shown a stronger

response to enrichment from lower quality substrates (Stelzer et al. 2003; Greenwood et al. 2007).

The rate at which basal resources provide energy and materials for higher trophic levels is a key component shaping food webs (Sterner et al. 1998). This rate is likely to be affected by nutrient enrichment via alterations in carbon:nutrient ratios. An increase in water column nutrients appeared to result in microbes, specifically fungi, taking up more P relative to N in the nutrient-enriched stream, presumably due to release from P-limitation (Fig. 5). For example, at a low C:N (e.g. 50), C:P in the reference stream would be predicted to be 2774. However, in the treatment stream, at the same C:N, we would predict that C:P would be 1166.

Additional losses of carbon are also likely to increase through respiration, as has been observed in terrestrial ecosystems (Mack et al. 2004). Our data suggest that nutrient enrichment will increase respiration associated with both fine and coarse detrital material. The greatest effect was observed on more recalcitrant substrates, which was also observed in a previous study in these same streams (Greenwood et al. 2007). Indeed, carbon budgets from the first three years of this study revealed significant increases in both microbial respiration and FPOM export with addition of nutrients (Benstead et al. 2009). Our results suggest that the response to chronic enrichment of aquatic ecosystems may differ not just among systems, but within systems as well, and varied responses among detrital types may drive divergent food web responses.

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Table 3.1. Results of two-way ANOVAs testing for effect of nutrient addition (Nutrient) on fungal biomass, bacterial biomass, microbial respiration, and C:N and C:P values, on FBOM, red maple, and rhododendron substrates. Date is treated as a fixed effect, and a significant interaction indicates that nutrient effects varied through time. NS = not significant ($p > 0.05$).

Fungal biomass	**FBOM**		**Maple**		**Rhododendron**	
Nutrient	$F_{1, 40} = 0.70$	NS	$F_{1, 18} = 20.65$	<0.001	$F_{1, 20} = 113.81$	<0.001
Date	$F_{9, 40} = 13.55$	<0.001	$F_{4, 18} = 20.01$	<0.001	$F_{4, 20} = 73.67$	<0.001
Nutrient x date	$F_{9, 40} = 0.68$	NS	$F_{3, 18} = 0.72$	NS	$F_{4, 20} = 9.25$	<0.001
Bacterial biomass						
Nutrient	$F_{1, 38} = 5.42$	0.025	$F_{1, 4} = 9.21$	0.039	$F_{1, 20} = 20.16$	<0.001
Date	$F_{9, 38} = 7.63$	<0.001	$F_{1, 4} = 15.17$	0.018	$F_{4, 20} = 11.72$	<0.001
Nutrient x date	$F_{9, 38} = 2.51$	0.023	$F_{1, 4} = 2.27$	NS	$F_{4, 20} = 4.77$	0.007
Microbial respiration						
Nutrient	$F_{1, 126} = 105.00$	<0.001	$F_{1, 16} = 9.83$	0.006	$F_{1, 20} = 5.71$	0.027
Date	$F_{17, 126} = 5.22$	<0.001	$F_{3, 16} = 2.95$	0.064	$F_{4, 20} = 3.74$	0.020
Nutrient x date	$F_{17, 126} = 1.94$	0.020	$F_{3, 16} = 7.37$	0.003	$F_{4, 20} = 6.58$	0.002
C:N						
Nutrient	$F_{1, 66} = 0.03$	NS	$F_{1, 12} = 172.16$	<0.001	$F_{1, 16} = 41.65$	<0.001
Date	$F_{9, 66} = 4.49$	<0.001	$F_{2, 12} = 6.18$	0.014	$F_{3, 16} = 14.94$	<0.001
Nutrient x date	$F_{9, 66} = 0.75$	NS	$F_{2, 12} = 1.80$	NS	$F_{3, 16} = 9.12$	<0.001
C:P						
Nutrient	$F_{1, 46} = 1.01$	NS	$F_{1, 12} = 112.70$	<0.001	$F_{1, 16} = 32.64$	<0.001
Date	$F_{6, 46} = 3.75$	0.004	$F_{2, 12} = 2.31$	NS	$F_{3, 16} = 2.18$	NS
Nutrient x date	$F_{6, 46} = 1.32$	NS	$F_{2, 12} = 0.47$	NS	$F_{3, 16} = 1.81$	NS

Table 3.2. Mean reference values for five different parameters on FBOM, red maple, and rhododendron substrates and percent change with enrichment. Percent change based on average of all samples in the reference stream compared to average of all samples in the treatment stream. Values in parentheses for C:N and C:P represent the maximum percent change occurring on a single sampling date.

	FBOM Reference	**FBOM % change**	**Red Maple Reference**	**Red Maple % change**	**Rhododendron Reference**	**Rhododendron % change**
Fungal biomass (mg C/g AFDM)	0.41	21	10.23	139	1.98	453
Bacterial biomass (mg C/g AFDM)	8.05	32	0.06	64	0.02	552
Microbial respiration (mg O2/g AFDM/hr)	0.10	52	0.17	122	0.04	303
C:N	19	3 (-72)	77	-36 (-86)	141	-28 (-120)
C:P	168	-17 (-116)	5188	-72 (-330)	8513	-54 (-415)

Table 3.3. Results of two-way ANCOVAs testing for effects on CPOM nutrient content. Fungal or bacterial biomass was used as the covariate and nutrient enrichment and leaf species as fixed effects.

Fungal biomass

	C:N			C:P		
CPOM	Fungi	$F_{1,34}$=25.63	p<0.001	Fungi	$F_{1,34}$=35.28	p<0.001
	Nutrients	$F_{1,34}$=16.28	p<0.001	Nutrients	$F_{1,34}$=82.70	p<0.001
	Leaf species	$F_{1,34}$=23.07	p<0.001	Leaf species	$F_{1,34}$=1751.49	p<0.001
	Nutrients x species	$F_{1,34}$=0.39	NS	Nutrients x species	$F_{1,34}$=47.75	p<0.001

Bacterial biomass

	C:N			C:P		
CPOM	Bacteria	$F_{1,27}$=20.42	p<0.001	Bacteria	$F_{1,27}$=13.81	p<0.001
	Nutrients	$F_{1,27}$=9.94	p=0.003	Nutrients	$F_{1,27}$=35.10	p<0.001
	Leaf species	$F_{1,27}$=23.51	p<0.001	Leaf species	$F_{1,27}$=5.14	0.032
	Nutrients x species	$F_{1,27}$=0.01	NS	Nutrients x species	$F_{1,27}$=0.98	NS

Table 3.4. Results of linear regression analyses of microbial biomass vs. nutrient content. These analyses were run based on results of two-way ANCOVAs testing for effects on CPOM nutrient content. NS = not significant, SE = standard error, Ref = reference stream, NP = treatment stream.

	p	Slope	SE	Intercept
Fungal biomass vs. C:N	<0.001	-0.23	0.03	5.04
Fungal biomass vs. C:P				
Maple Ref	NS	-0.09	0.07	8.65
Maple NP	0.031	-0.19	0.06	7.86
Rhododendron Ref	NS	-0.03	0.07	8.87
Rhododendron NP	<0.001	-0.25	0.03	8.73
Bacterial biomass vs. C:N	<0.001	-0.22	0.04	3.88
Bacterial biomass vs. C:P	<0.001	-0.34	0.08	7.14

Table 3.5. C:nutrient TERs and nutrient ratios of associated food resources for shredders and collector-gatherers in the reference and treatment streams(from Cross et al. 2003). Signs indicated whether the TER is above or below the associated resource ratio (+ indicates C limitation; - indicates N or P limitation).

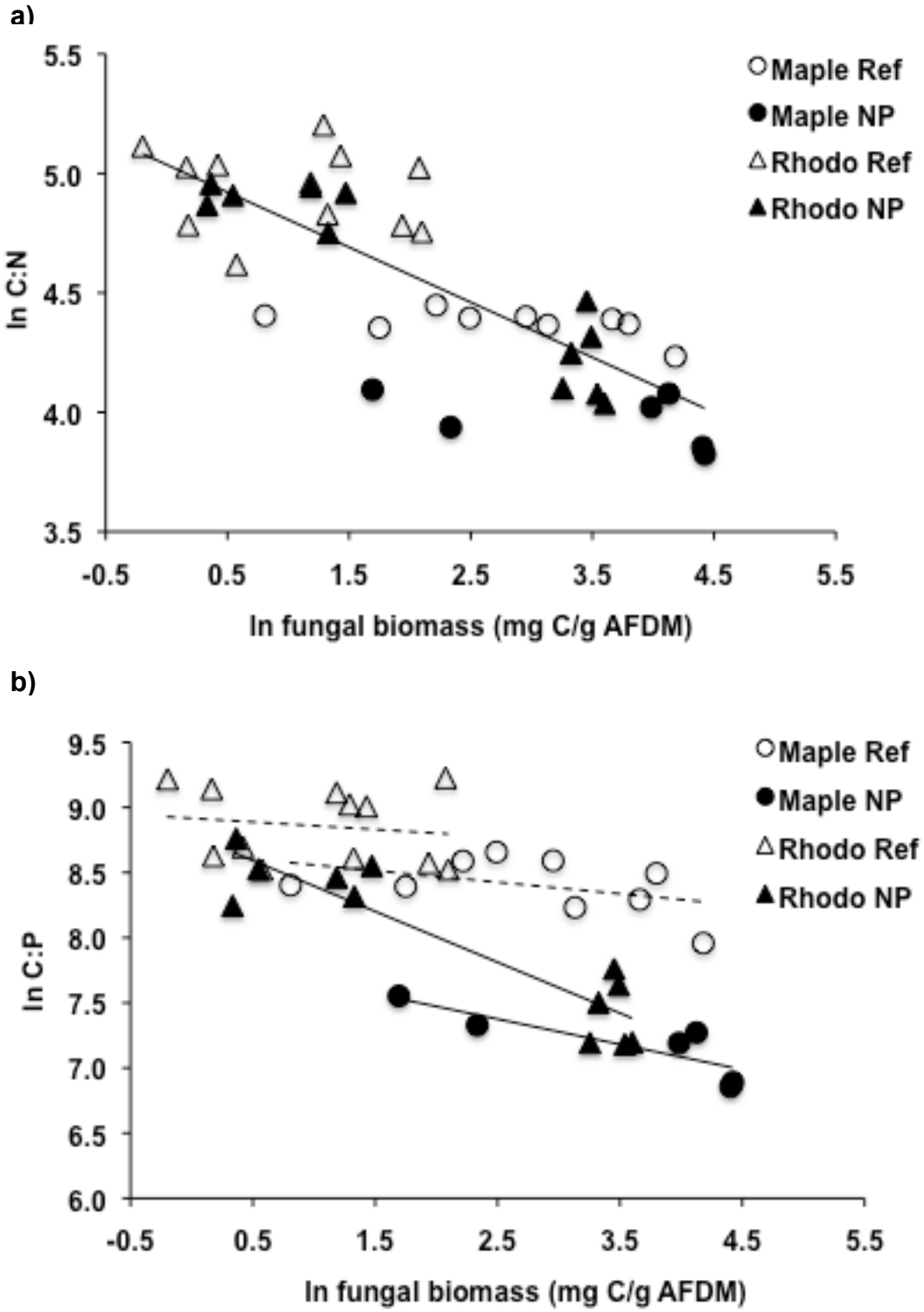
TER			Food resource		
	Reference	Treatment		Reference	Treatment
Shredders			CPOM		
C:N	27 (-)	26 (-)	C:N	57	55
C:P	1992 (-)	1008 (-)	C:P	4854	3063
Collectors			FPOM		
C:N	26 (+)	24 (+)	C:N	19	20
C:P	1108 (+)	908 (+)	C:P	168	140

Fig. 3.1. Relationship between CPOM fungal biomass and substrate a) C:N and b) C:P ratios. Solid regression lines indicate nutrient-enriched treatments. Dashed lines indicate reference treatments.

Fig. 3.2. Relationship between CPOM bacterial biomass and substrate a) C:N and b) C:P ratios.

Fig. 3.3. Relationship between substrate C:N and C:P ratio on a) CPOM (reference: $p < 0.001$, $r^2 = 0.72$, $y = 0.93x + 4.29$; nutrient: $p < 0.0001$, $r^2 = 0.96$, $y = 1.46x + 1.35$) and b) FBOM (reference: $p < 0.001$, $r^2 = 0.77$, $y = 2.32x – 2.00$; nutrient: $p < 0.0001$, $r^2 = 0.85$, $y = 1.95x – 1.13$). Solid regression lines indicate nutrient-enriched treatments. Dashed lines indicate reference treatments.

Fig. 3.1.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ln C:N
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0.5
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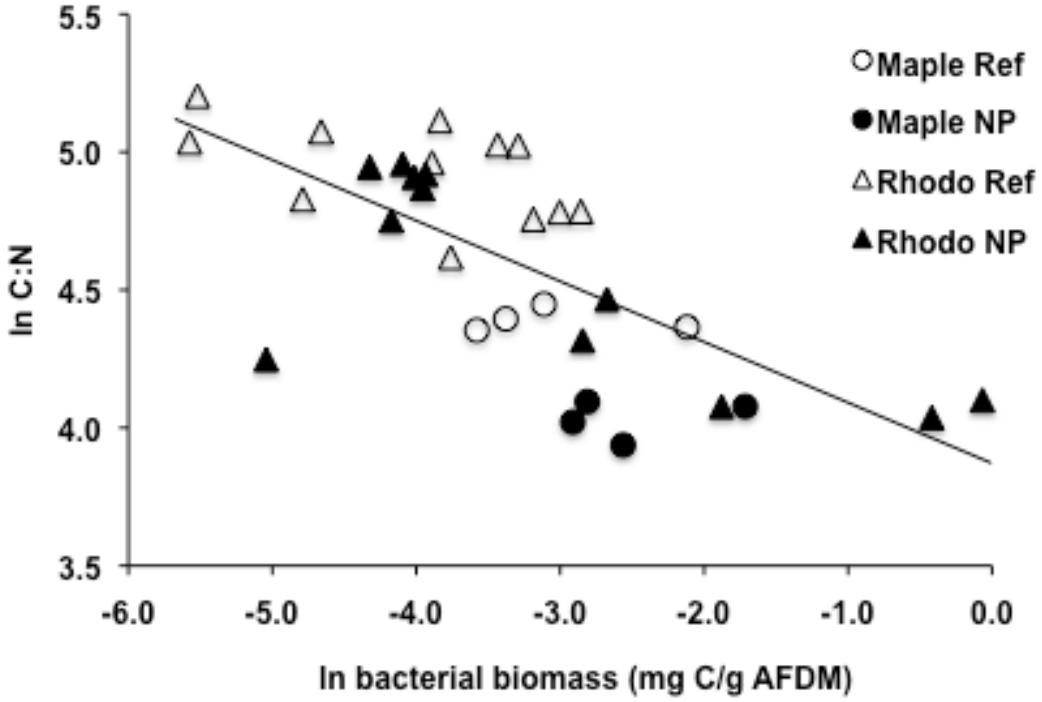
4.5
5.5
ln fungal biomass (mg C/g AFDM)
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP
b)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
ln C:P
-0.5
0.5
1.5
2.5
3.5
4.5
5.5
ln fungal biomass (mg C/g AFDM)
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP

Fig. 3.2.

a)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ln C:N
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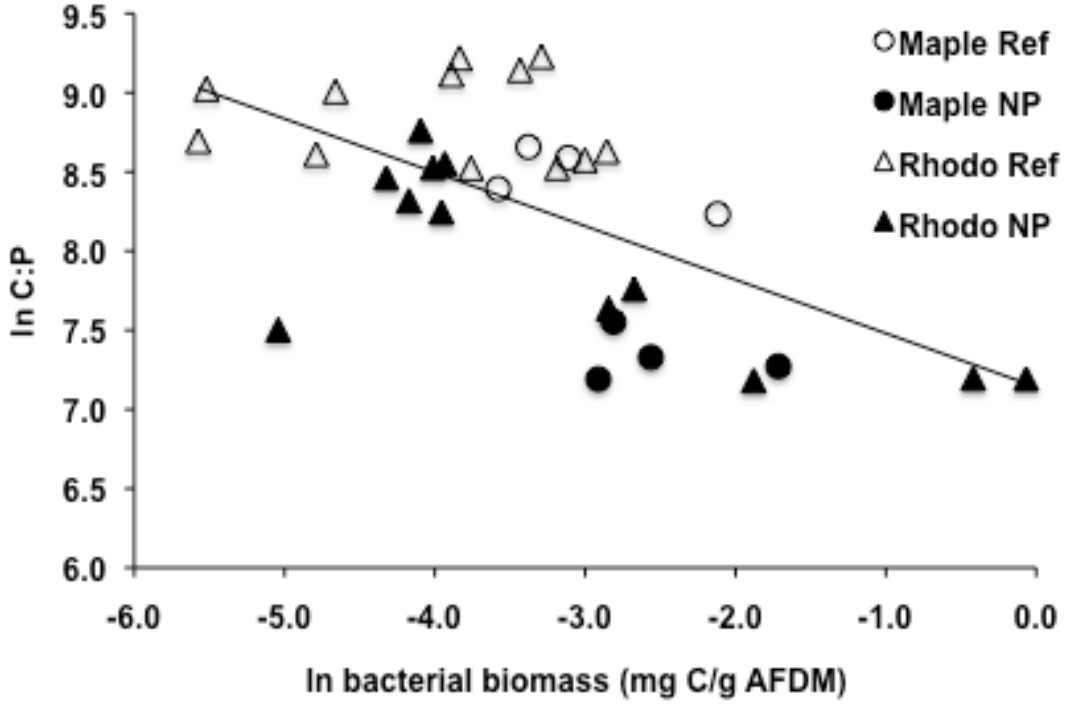
ln bacterial biomass (mg C/g AFDM)
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP

b)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ln C:P
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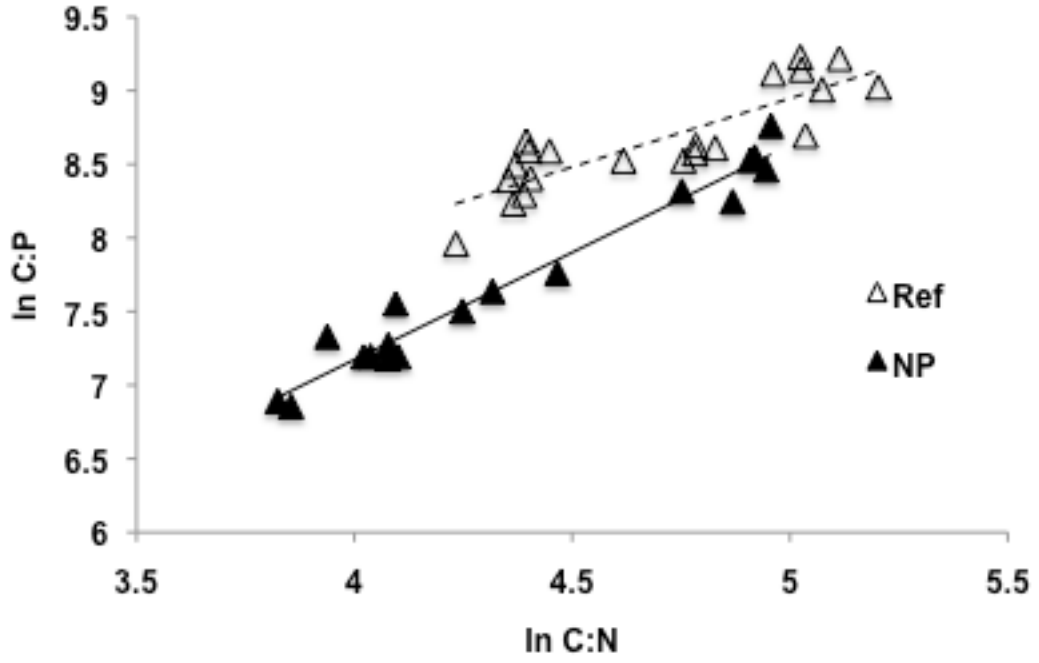
0.0
ln bacterial biomass (mg C/g AFDM)
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP

Fig. 3.3.

a)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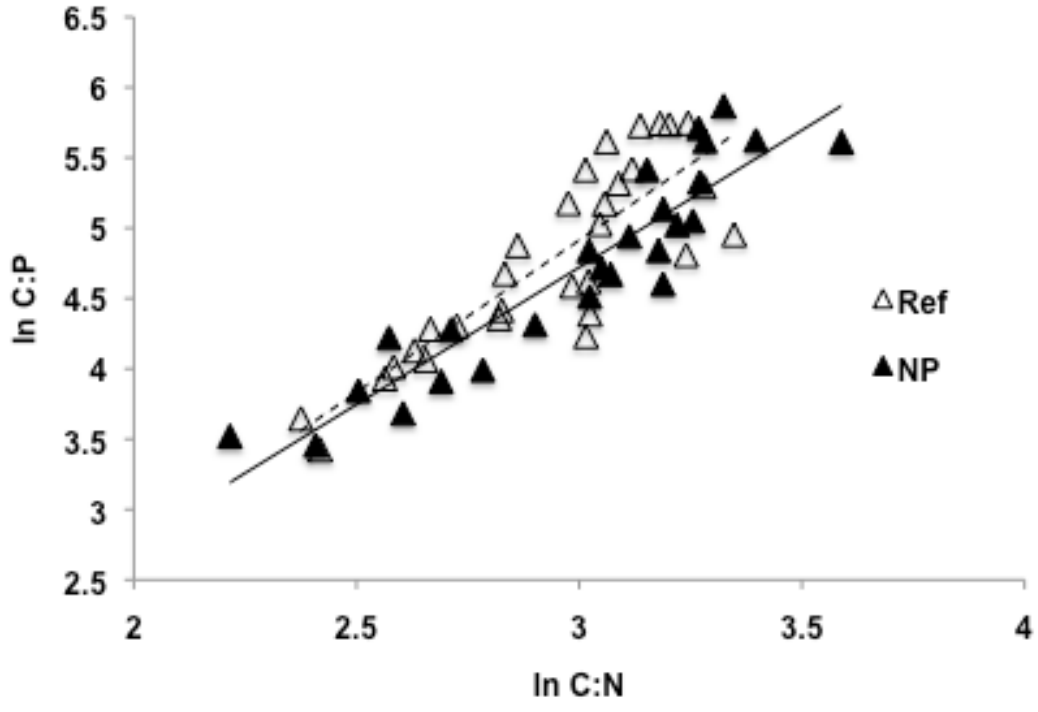
ln C:N
Ref
NP

b)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4.5
4
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3
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ln C:P
2
2.5
3
3.5
4
ln C:N
Ref
NP

CHAPTER 4

THE ROLE OF AQUATIC FUNGI IN NUTRIENT-MEDIATED ORGANIC MATTER TRANSFORMATIONS[3]

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Abstract

Here we assessed the key role of aquatic fungi in modifying coarse particulate organic matter (CPOM) by affecting its breakdown rate, nutrient content, and conversion to fine particulate organic matter (FPOM). We hypothesized that fungal-mediated transformations of CPOM would be accelerated under elevated nutrient concentrations and tested whether fungal presence was critical to CPOM processing rates and FPOM production by an invertebrate consumer. We manipulated the presence and absence of fungi, exogenous nutrients, and an invertebrate consumer in a full factorial laboratory experiment and quantified their effects on CPOM mass loss and nutrient content, and the quantity and nutrient content of FPOM produced during leaf breakdown. Breakdown rates of CPOM and the quantity of FPOM produced were highest in nutrient amended treatments containing fungal decomposers. We found significant interactions between nutrients and fungi on most response variables, indicating that nutrient effects on organic matter transformations were dependent on the presence of fungal decomposers. We observed linear increases in CPOM nutrient content related to fungal biomass and a non-linear increase in mass loss of CPOM with fungal biomass in the presence of shredders. In contrast, fungal effects on FPOM nutrient content were less predictable, whereby fungi decreased FPOM P content, but did not significantly effect its N content. These results indicate that aquatic fungi play a critical role in facilitating energy and nutrient flow through both CPOM and FPOM food web pathways and that their ability to mediate organic matter transformations are significantly influenced by nutrient enrichment.

Introduction

Heterotrophic microbes are widely recognized for their role in mediating the conversion and transfer of energy and nutrients to higher trophic levels in many detrital-based ecosystems (Moore et al. 2004). Many diverse ecosystems are detritus-based and depend on terrestrial inputs of organic matter as the major source of carbon and nutrients for the food web (Wallace et al. 1997; Sundareshwar et al. 2003; Mack et al. 2004). As the dominant group of microorganisms on coarse particulate organic matter (CPOM) (Findlay et al. 2002), fungi may contribute significantly to the resource quality of these fractions for consumers. Thus, they may also affect downstream food webs via transformation and conversion of CPOM fractions into fine particulate organic matter (FPOM). As a result, elucidating the role of fungi is key to understanding how alterations in resource availability (e.g. carbon and nutrients) potentially affect underlying processes in detritus-based systems.

Detritus-based aquatic ecosystems currently face multiple stressors, including loss of or changes in the composition of riparian vegetation and other detrital subsidies (Ellison et al. 2005), as well as nutrient enrichment resulting from atmospheric deposition or runoff from the terrestrial landscape (Smith and Schindler 2009). Microbial decomposers, associated with organic matter, likely respond to these environmental stressors, which in turn may alter organic matter processes and food web dynamics. Given the important role of heterotrophic microbes in organic matter transformations and associated changes in detrital resource quality, knowledge of their responses to environmental stressors and their influence on aquatic food webs is needed to better predict the impacts of environmental change. The response by microbial colonists of CPOM (e.g., fungi) are likely particularly important, as they are the 'first line' in transformations of detritus entering food webs. However, we currently have a limited understanding of the

specific responses of fungi and other heterotrophic microbes in 1) their responses to nutrient enrichment and 2) their overall contributions to organic matter processing within altered ecosystems.

Specifically, microbial response to nutrient enrichment may not only affect CPOM quality directly, but may also affect FPOM quality and quantity via consumption pathways by invertebrate detrital consumers. Heterotrophic microorganisms provide the initial pathway by which nutrients are taken up from the water column (Gulis and Suberkropp 2003c). Because microorganisms are higher in nutrient content than the detrital substrates they colonize, their presence and growth increases nutrient content of the microbial/detrital complex (Stelzer et al. 2003), which is critical for the growth of detritivores (Arsuffi and Suberkropp 1985; Barlocher 1985; Chung and Suberkropp 2009). As a consequence, nutrient enrichment may facilitate the increased generation of FPOM due to increased fecal egestion from feeding activities, thereby having indirect effects on organisms that feed on fine fractions of detritus (Pandian and Marian 1986; Wallace and Webster 1996).

A large-scale experimental nutrient enrichment of a stream ecosystem has shown increased flow of nitrogen and phosphorus to detrital consumers, which presumably occurred via microbial nutrient uptake and immobilization, and subsequent consumption of microbial-colonized detritus (Cross et al. 2007). Fungi have been implicated as the primary driver of this process, as biomass and production of heterotrophic microbes, particularly fungi, increased under nutrient enrichment (Gulis et al. 2008; Suberkropp et al. 2010). In addition, nutrient enrichment also resulted in increased ecosystem-scale loss rates of carbon due to increases in microbial respiration and FPOM export compared to a reference stream (Benstead et al. 2009). Increased FPOM export was likely a result of the positive microbial response to enrichment, which directly

facilitated CPOM mass loss and FPOM generation through microbial-mediated invertebrate consumption of CPOM. However, given the difficulties associated with quantifying the functional role of heterotrophic microbes in an ecosystem-level experiment, their specific role in these ecosystem-level responses has not been previously quantified.

In the present study, we conducted a controlled laboratory experiment to more closely examine the underlying mechanisms driving increased rates of conversion of CPOM to FPOM under enriched conditions and distinguish nutrient enrichment effects on CPOM and FPOM. Specifically, we sought to quantify and test the role of fungi and interactions with consumers in driving the previously observed ecosystem-level effects of FPOM generation (Benstead et al. 2009). We manipulated the presence or absence of fungi, nutrients, and invertebrate consumers in a full factorial laboratory experiment that quantified the effect of fungal presence on four aspects of organic matter transformation under nutrient enrichment. These experiments tested if fungal presence influenced: 1) the nutrient content of CPOM; 2) CPOM mass loss (i.e. leaf breakdown); 3) the quantity of FPOM produced via invertebrate feeding; 4) the nutrient content of FPOM. Our studies used biota from the same reference stream at the Coweeta Hydrologic Laboratory (CHL), North Carolina, USA, used in the ecosystem-scale studies cited above.

Overall, we expected that fungi would be critical in mediating organic matter transformations under increased nutrient enrichment. At the extreme, we predicted that fungal presence would be necessary for any effects of nutrient enrichment on CPOM to be manifested, which would not occur with bacteria alone. Specifically, we predicted that CPOM mass loss and FPOM production would be greatest in the presence of elevated nutrients and aquatic fungi. We also predicted that the combination of nutrients and aquatic fungi would have the greatest impact on CPOM and FPOM nutrient content.

Methods

Experimental Design

The experimental design consisted of three factors at two levels: fungi reduced (-) or present (+), nutrients ambient (-) or moderately enriched (+), and shredders absent (-) or present (+). A complete randomized block design was used. The treatments were as follows: 1) - fungi/- nutrients, 2) - fungi /+ nutrient, 3) + fungi / - nutrients, 4) + fungi /+ nutrient. Furthermore, to evaluate the interactive effects of shredding invertebrates, the four treatments above were crossed with presence or absence of an invertebrate shredder. Microcosms containing sterilized leaf discs within ambient or nutrient-enriched (nitrogen (N) and phosphorus (P) at moderate concentrations) stream water were inoculated with a 'fungi-reduced' or a 'fungi-included' microbial inoculum and allowed to incubate for 14 days (see below). After 14 days, individuals of the genus *Pycnopsyche* (Trichoptera, Limnephilidae) were added to half of the microcosms and allowed to feed on leaf discs for 72 hours. We selected this taxon because it is a leaf-shredding consumer commonly found in streams of temperate deciduous forests (Ross 1963) and is a primary driver of organic matter processing in such ecosystem types (Creed et al. 2009). Sterilized leaf disc controls (no inoculum) were used to estimate leaf mass loss due to leaching.

Laboratory Microcosms

Red maple (*Acer rubrum* L.) leaves were collected at CHL in the fall of 2007 after abscission, returned to the laboratory, and allowed to air dry. Leaf discs were cut (11.8 mm diameter), soaked in deionized water for 48 hours, dried at 60ºC for 48 hours, and then sterilized with gamma irradiation (2 megarads applied over 5 days). Sets of 20 sterilized leaf discs were weighed for initial mass before being added to each microcosm. We added 105 sets of 20 sterile leaf discs to microcosms (100 mL plastic beakers) containing 80 mL of either filtered stream

water or filtered stream water amended with N and P at targeted concentrations of 96 μg L^{-1} SRP and 500 μg L^{-1} dissolved inorganic nitrogen (from stock solutions of NH_4NO_3 and KH_2PO_4 + K_2HPO_4). These concentrations are within the range of concentrations observed in streams experiencing land use change in the Southern Appalachians (Scott et al. 2002) and were within the range of concentrations used in the whole-stream nutrient enrichment experiment (above). Microcosms were incubated in an experimental growth room at 15ºC on a 12/12 hr light/dark cycle. Stream water for the study was collected from stream 53 at CHL (hereafter referred to as the reference stream) and filter-sterilized using 0.22 μm Isopore membrane filters (Millipore).

To obtain the microbial inocula, red maple leaves were collected from the reference stream and homogenized in a blender with filtered stream water. The resulting slurry was allowed to settle for several minutes and a portion filtered through a 3μm Whatman Nuclepore membrane filter. This filtrate was used as our inoculum for 'fungi-reduced' treatments. The remaining unfiltered portion of this slurry was used as the inoculum for 'fungi-included' treatments. A subsample of both the filtered and unfiltered slurry was filtered through a membrane filter (8 μm pore size, 25 mm diameter, Millipore, USA) and stained with 0.1% trypan blue in lactic acid to estimate number of fungal spores. Two mL aliquots of corresponding microbial inocula were added to each treatment. Microbial inocula for the 'fungi-included' treatment contained fungal spores at a concentration of approximately 3400L^{-1}. Despite efforts to completely remove all fungi from the 'fungal-reduced' treatments, occasional spore fragments remained after filtration. However, fungal biomass remained very low in these treatments, and filtration at a smaller pore size may have removed non-target components of the microbial inoculum (i.e. bacteria).

All microcosms were aerated for 14 days. Microcosm water was replaced every 72 hours. After 14 days, five replicates from each treatment were removed to determine leaf litter mass loss, litter-associated fungal biomass, and litter carbon (C), N, and P content prior to the addition of *Pycnopsyche*. To examine the interactive effect of nutrients and fungi on *Pycnopsyche* shredding activity and FPOM production, the remaining replicates within each treatment were incubated an additional 72 hours in either the presence or absence of *Pycnopsyche*. Final instar *Pycnopsyche* individuals (in stone cases) were collected from the CHL reference stream and transported back to the laboratory. Individuals were acclimated in aquaria for several days in the experimental growth room at 15ºC and starved for 48 hours prior to being added to the microcosms. After the two-week incubation period, individual *Pycnopsyche* were added to half of the treatment microcosms and allowed to feed for 72 hours. After 72 hours of feeding, any CPOM remaining was removed from each microcosm, and *Pycnopsyche* were allowed to clear their guts for 96 hours and were then removed from each microcosm.

Sample Analyses

To determine mass loss of CPOM and mass of FPOM generated, remaining leaf particles (CPOM, >1 mm) were manually removed with forceps from each microcosm, and FPOM (<1 mm) was filtered through a 0.7 µm glass fiber filter. Both were dried at 60ºC and weighed. Material was then ground in a ball mill and analyzed for C and N content with a Carlo Erba NA 1500 CHN analyzer. Phosphorus content was analyzed spectrophotometrically after acid digestion (APHA 1998).

Litter-associated fungal biomass was estimated from concentrations of ergosterol in plant litter (Gessner 2005). Five leaf discs from each replicate were placed into 20 ml plastic

scintillation vials, preserved with 5 mL of HPLC grade methanol, and stored at -20˚C until extracted. Ergosterol in samples was extracted in alcoholic KOH (0.8% KOH in methanol, total extraction volume 10 ml) for 30 minutes at 80ºC in tightly capped tubes with constant stirring. The resultant crude extract was partially cleaned by solid phase extraction (Gessner and Schmitt 1996), and ergosterol quantified by high-pressure liquid chromatography (HPLC). An HPLC (LC-10AT pump and SPD-10A UV-VIS detector, Shimadzu Scientific Inc.) was used for separation and analysis of ergosterol. The mobile phase was HPLC grade methanol at a flow rate of 1.5 mL min^{-1}. Ergosterol was detected at 282 nm (retention time = ~ 7.5 min) and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical Co.). Ergosterol concentrations were converted to fungal biomass assuming an ergosterol concentration of 5.5 µg mg^{-1} of mycelial dry mass (Gessner and Chauvet 1993).

Statistical Analyses

To quantify the proportionate change on measures of quality and quantity resulting from the presence of fungi, the magnitude of response was determined under both nutrient and reference conditions (Hedges et al. 1999). The response ratio, RR, was calculated by comparing means for fungi present treatments with fungi absent treatments:

$$RR = (+fungi)\ (-fungi)^{-1}$$

Response ratios were calculated for CPOM nutrient content prior to shredder feeding (to avoid effects of selective feeding by *Pycnopsyche*), and CPOM mass loss, FPOM production, and FPOM nutrient content were all analyzed from post-feeding treatments. Response ratios greater than one indicate a positive fungal effect.

To test for treatment effects on fungal biomass and leaf nutrient content prior to shredder feeding, a two-way analysis of variance (ANOVA) was used to test for main and interactive

effects of fungi and nutrients on ergosterol and CPOM N and P content after the initial 14-day incubation period. These analyses examined the role of nutrients and fungi in driving initial CPOM quality. A three-way ANOVA was used to test for the main and interactive effects of nutrients, fungi, and shredders in driving mass loss from CPOM and mass accrual of FPOM. A post-feeding two-way ANOVA was conducted on N and P content of FPOM (from the shredder present treatments) to test for nutrient and fungal effects on FPOM nutrient content. A Tukey-Kramer multiple comparison test was used to test for differences among treatments when significant main or interactive effects of ANOVAs were found.

Relationships between fungal biomass and nutrient content of CPOM were also examined with regression analyses. Data were transformed where necessary to improve normality and reduce heteroscedasticity. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC 27513, USA).

Results

Initial fungal biomass

There were significant fungal and nutrient effects, as well as a significant nutrient × fungal interaction on the quantity of fungal biomass that colonized leaf discs during the initial 14-day experimental period (Table 4.1). Fungal biomass was significantly higher in the fungi + nutrient treatments than in any other treatment (Tukey-Kramer $p < 0.0001$, Table 4.2).

Effects of nutrients and fungi on organic matter quantity

CPOM mass loss was significantly affected by fungi, nutrients, and shredders, as well as nutrient × fungi and nutrient × shredder interactions (Table 4.3). CPOM mass loss was highest in treatments with fungi, nutrients, and shredders (Tukey-Kramer $p < 0.0001$; Fig. 4.1A). Mass loss in shredder-absent treatments was attributed to leaching and microbial processing of leaf

material (e.g. microbial respiration and leaf softening/fragmentation), particularly in the treatments containing both fungi and nutrients. Fungi, nutrients, and shredders also significantly affected the quantity of FPOM produced, and there were significant interactions among all three factors (Table 4.3). FPOM produced was significantly higher in nutrient treatments (Tukey-Kramer $p < 0.0001$; Fig. 4.1B) and with fungal presence (Tukey-Kramer $p < 0.0001$). When comparing the effects of fungi within either the ambient or nutrient-enriched treatments, fungal presence had a positive effect on both CPOM mass loss (45% increase) and FPOM production (85% increase) at elevated nutrient levels, but negligible effects at ambient nutrient concentrations (Fig. 4.2A).

Effects of nutrients and fungi on initial CPOM quality

Both the presence of fungi and exogenous nutrients affected the initial N content of CPOM (Table 4.1), as measured after the 14 day incubation before shredder feeding. Percent N of CPOM was significantly higher in the fungi + nutrient treatments than in all other treatments (Tukey-Kramer $p < 0.0001$; Fig. 4.3A). Initial P content was also significantly higher in the nutrient enriched treatments (ANOVA, $F_{1,16} = 20.71$, $p = 0.0003$); however, this increase was not dependent on fungal presence (Table 4.1, Fig. 4.3B). Prior to shredder feeding, fungal presence positively affected N content (30% increase) and only slightly increased P content (9% increase) of CPOM that was exposed to elevated nutrient levels. Under ambient nutrient conditions, fungal presence had either no effect (N content) or a negative effect (P content) on CPOM nutrient content (Fig. 4.2B).

Effects of nutrients and fungi on FPOM quality

FPOM produced by shredders had significantly higher N content in nutrient treatments than reference treatments (Tukey-Kramer $p < 0.0001$; Table 4.4; Fig. 4.3C), but the effect did not

increase in the presence of fungi (Tukey-Kramer $p = 0.287$; Fig. 4.2C). Phosphorus content of FPOM was significantly lower in fungi + nutrient treatments than any other treatment (Tukey-Kramer $p = 0.037$; Fig. 4.3D). Fungal presence reduced FPOM P content by 42% (Fig. 4.2C).

Relationship between fungal biomass and CPOM characteristics

In addition to response ratios, slopes of regression lines also allow us to quantify how much change in organic matter occurred due to fungal presence, but here the relationship can be determined over a gradient rather than presence/absence. Fungal biomass was significantly positively related to CPOM N content ($p < 0.0001$; $r^2 = 0.86$; Fig. 4.4A). The relationship between fungal biomass and P content was significant but more variable than with N ($p = 0.0157$; $r^2 = 0.24$; Fig. 4.4B). To evaluate the contribution of shredders to fungal-mediated mass loss, we constructed separate relationships between mass loss and fungal biomass, with and without shredders. Mass loss increased with fungal biomass for both shredder and no shredder treatments, but CPOM mass loss leveled off with higher fungal biomass in the presence of shredders (Fig. 4.5). A more linear relationship was observed in the absence of shredders, suggesting that the fundamental relationship of fungal biomass and mass loss was altered by the presence of shredders (Fig. 4.5).

Discussion

Our results highlighted the connection between fine and coarse organic matter, yet isolated differences in nutrient content of the two detrital types as a result of nutrient enrichment. These data also highlight the importance of aquatic fungi in facilitating organic matter transformations via shredding macroinvertebrates. Moreover, data obtained in the present study support prior field-based research (Gulis et al. 2008; Suberkropp et al. 2010) indicating that the contribution and impact of fungal decomposers on detrital processes are increased under

nutrient-enriched conditions and that fungal decomposers are a key driver of organic matter transformations at high nutrient availability. Under field conditions, a positive fungal response (e.g. biomass, production, sporulation, etc.) to increased nutrient concentrations has been observed, and this response has been associated with increased organic matter breakdown rates (Suberkropp and Chauvet 1995; Gulis and Suberkropp 2003c; Ferreira et al. 2006). Furthermore, positive impacts of both shredders and nutrients on downstream export of FPOM have been demonstrated (Wallace et al. 1991; Benstead et al. 2009). However, identifying causal mechanisms influencing microbial and invertebrate processes in response to nutrient enrichment and their potential interactions is often difficult to decipher in watershed-scale field studies. As a consequence, controlled laboratory manipulations provide useful opportunities to more closely examine potential effects of nutrients on microbes and invertebrates and their potential interactions. Results of our microcosm experiment suggest that, under nutrient enrichment, fungi are explicitly linked to both losses of CPOM and increased production of FPOM via increased detrital palatability and shredder feeding activity.

Elevated nutrients supported the increased growth of litter-associated fungi in our microcosms, which has also been previously observed in both field (Gulis and Suberkropp 2003c; Stelzer et al. 2003; Ferreira et al. 2006; Suberkropp et al. 2010) and controlled laboratory studies (Gulis and Suberkropp 2003a). In comparison to fungi-reduced treatments, the presence of fungi in nutrient-enriched treatments increased CPOM mass loss by only 23% when *Pycnopsyche* were excluded. In contrast, CPOM mass loss was 43% higher than fungi-reduced treatments when both fungi and *Pycnopsyche* were present in nutrient-enriched treatments. Similarly, the quantity of FPOM generated by *Pycnopsyche* nearly doubled in the presence of fungi and elevated nutrients, as compared to the fungal-reduced treatments. This suggests that

fungi facilitated *Pycnopsyche*'s role in organic matter transformation. In this experiment, increased mass loss in microcosms with fungi but no shredders was likely due to microbial conditioning. Because fungi, as well as bacteria, have much lower carbon:nutrient ratios than the substrates that they colonize, their colonization of these substrates creates a resource of higher nutritional value for consumers (Cummins and Klug 1979; Stelzer et al. 2003). Thus fungi, both by immobilizing nutrients directly from the water column and through extra-cellular enzymatic degradation, transform relatively recalcitrant detritus into a more palatable form for consumers (Barlocher 1985; Gessner and Chauvet 1994; Suberkropp and Chauvet 1995).

Our data suggest that fungal colonization of leaf material facilitated organic matter transformations by detritivores, subsequently affecting nutrient and energy flow in CPOM and FPOM food web pathways. Shredding macroinvertebrates have been previously cited as important drivers of organic matter transformations of CPOM to FPOM (Wallace et al. 1991). Prior evidence has shown that shredder feeding on leaf detritus may create a 'processing chain' whereby increased fecal egestion by shredders (i.e., FPOM) can facilitate collector-gatherer feeding through this increase in available FPOM (Cummins 1973; Short and Maslin 1977; Heard 1994). Because the presence of fungi increased FPOM production associated with shredder feeding activities, our study suggests that fungi are an integral first step in this processing chain and help facilitate the role of shredders in increasing the availability of FPOM for other stream consumers.

Despite fungi increasing the availability of FPOM, the combined effects of fungi and nutrients did not appear to trickle down to FPOM nutrient content during organic matter transformations. While N content of FPOM did increase in the presence of elevated nutrients, fungi had no effect, and P content of FPOM was significantly lower in the presence of fungi and

elevated nutrients. Although *Pycnopsyche* increased the quantity of FPOM produced, they had a much lower effect on FPOM nutrient content, even in enriched conditions. Because increased resource quality can increase the nutrient assimilation efficiency of consumers (Pandian and Marian 1986), the reduced P content of FPOM may have been a result of shredders increasing their assimilation efficiency of limiting nutrients, specifically P, in elevated nutrient treatments. Therefore, increased CPOM palatability that enhanced shredder assimilation of carbon and nutrients from CPOM may indirectly reduce the quality of FPOM produced as feces. This reduction in FPOM nutrient content has the potential to substantially alter trophic flows within FPOM food web pathways, subsequently affecting those consumers that rely on FPOM (i.e. collector-gatherers). Consumers in our study streams may be primarily P-limited (Cross et al. 2003), so these reductions in FPOM P content have the potential to affect consumer production more than the increases in FPOM N content. For instance, collector-gatherers repeatedly re-ingest FPOM in the form of feces, reducing the likelihood that their production will be limited by FPOM availability. Specifically, collector-gatherer secondary production can be higher than FPOM standing crop, suggesting that collector-gatherer production requires rapid turnover and re-ingestion of FPOM (Fisher and Gray 1983; Romito et al. 2010). If fungi indirectly reduce P content and the initial quality of FPOM generated under nutrient-enriched conditions, as collector-gatherers repeatedly re-ingest this lower quality resource, this potential nutrient limitation may become exacerbated through time.

Some effects of nutrients on organic matter transformations were also observed in treatments where fungal biomass was reduced. Even when fungal biomass was reduced, elevated nutrients still resulted in a 25% increase in CPOM mass loss and a 43% increase in FPOM produced when shredders were present. These results suggest the potential positive

influence of leaf degrading bacteria (i.e. biofilms) on detritivore feeding. Although we did not assess bacterial biomass in the present study, prior laboratory experiments have reported a positive response of bacteria to elevated nutrients in the absence of fungi (Gulis and Suberkropp 2003b). Nutrient amendments also affected the nutrient content of CPOM and FPOM in fungal-reduced treatments. There was no clear effect of fungal presence on P content of CPOM prior to *Pycnopsyche* feeding. However, nutrient amendments did increase P content by 62% in fungal-reduced treatments and by 200% in fungal-present treatments when compared to reference conditions.

The lack of a measurable effect of fungi in ambient conditions is likely a result of the short incubation time of this experiment (e.g. 14 days). Because the focus of the experiment was on nutrient-enriched conditions, peaks in fungal biomass under enriched conditions, which occur well before peaks under ambient conditions, were targeted (Gulis and Suberkropp 2003c). As fungal biomass under reference conditions peaks at a later date (Gulis and Suberkropp 2003c), it is possible that fungi may have performed a similar role in nutrient mobilization under reference conditions if provided with longer incubation times. Effects of similar magnitude may occur in the presence and absence of nutrient enrichment, but these effects may occur on a very different temporal scale.

Our results are limited to a single shredder species transforming detritus from a single leaf species. Red maple and *Pycnopsyche* were chosen for this study due to their prevalence in streams in the eastern US. Shredders, such as *Pycnopsyche* studied here, play a major role in CPOM breakdown and the production of FPOM in streams (Wallace et al. 1991; Creed et al. 2009), and both shredder identity and leaf litter species composition may affect the quantity and quality of FPOM in streams (Balseiro and Albarino 2006). Different shredder species that are

consuming different leaf types might create FPOM with diverse nutritional characteristics. More work with other leaf species and shredders species would further elucidate these interspecific effects. Fecal material from aquatic insects is both a viable and abundant source of FPOM for collectors; therefore, these transformations of leaf material are important sources of energy and nutrients for these organisms in stream ecosystems (Shepard and Minshall 1984a; Shepard and Minshall 1984b).

The role of aquatic fungi in donor-controlled streams is important locally as a highly nutritious food resource for consumers, but can also affect downstream food webs via contributions to organic matter transformations. Through quantification of the role of fungi in these processes, our study suggests that this function is even more important in streams where inorganic nutrient concentrations are elevated. The prevalence of detritus-based stream systems and the widespread nature of eutrophication make it imperative that we understand the mechanisms driving the response to nutrient enrichment in streams that are net heterotrophic and/or that occur via heterotrophic pathways. An understanding of the complex nature of microbial responses to nutrient enrichment and their effects in a variety of stream ecosystem types are necessary prerequisites for proper management with a goal of restoring and/or preserving ecosystem services on which human populations depend.

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Table 4.1: Two-way ANOVA results of main and interactive effects of fungi and nutrients on fungal biomass, % N, and % P of CPOM prior to shredder feeding (ns = not significant).

		Ergosterol		Nitrogen		Phosphorus	
Source of variation	df	*F*	*p*-value	*F*	*p*-value	*F*	*p*-value
Fungi	1, 16	115.47	<0.0001	49.39	<0.0001	0.13	ns
Nutrient	1, 16	93.31	<0.0001	187.51	<0.0001	20.71	0.0003
Fungi x nutrient	1, 16	83.73	<0.0001	53.55	<0.0001	0.83	ns

Table 4.2: Mean fungal biomass (μg g dry wt $^{-1}$; n = 5) associated with CPOM after the initial 14-day incubation period and prior to shredder feeding in nutrient-enriched and reference treatments. Standard errors are in parentheses. A significant difference in treatments is indicated by an asterisk (ANOVA $p < 0.05$).

	No fungi	Fungi
Reference	4.31 (2.64)	7.24 (3.23)
Nutrient	5.25 (3.34)	41.75 (6.23)*

Table 4.3: Three-way ANOVA results of main and interactive effects of fungi, nutrients, and shredders on CPOM mass loss and FPOM production following the shredder feeding period (ns = not significant).

		CPOM Mass loss		FPOM produced	
Source of variation	df	*F*	*p*-value	*F*	*p*-value
Fungi	1, 84	19.37	<0.0001	14.25	0.0003
Nutrient	1, 84	86.23	<0.0001	45.61	<0.0001
Shredder	1, 84	282.65	<0.0001	217.46	<0.0001
Fungi x nutrient	1, 84	19.36	<0.0001	13.65	0.0004
Fungi x shredder	1, 84	1.81	ns	6.69	0.0117
Nutrient x shredder	1, 84	7.14	0.0093	19.52	<0.0001
Fungi x nutrient x shredder	1, 84	0.20	ns	3.64	0.0605

Table 4.4: Two-way ANOVA results of main and interactive effects of fungi and nutrients on % N and % P of FPOM produced in treatments with shredders present (ns = not significant).

		Nitrogen		Phosphorus	
Source of variation	df	*F*	*p*-value	*F*	*p*-value
Fungi	1, 16	1.30	ns	4.50	0.0259
Nutrient	1, 16	43.43	<0.0001	5.25	0.0385
Fungi x nutrient	1, 16	2.93	ns	3.49	0.0672

Figure Legends

Fig. 4.1. (A) Coarse particulate organic matter (CPOM) mass loss and (B) Fine particulate organic matter (FPOM) produced (mean ± 1SE) following shredder feeding (72 hours) in treatments with and without shredders, nutrients, and fungi (n = 15 in shredder treatments, n = 5 in no shredder and control treatments). Letters indicate significant differences in treatments (ANOVA $p < 0.05$).

Fig. 4.2. Response ratios of fungi on A) CPOM mass loss and FPOM production in treatments with shredders present, B) CPOM N content and P content prior to shredder feeding and, C) N content and P content of FPOM produced by shredders. Values greater than one indicate a positive fungal effect. Other designations as in Fig. 4.1.

Fig. 4.3. Effects of nutrients and fungi on nutrient content of CPOM and FPOM. Percent (A) nitrogen and (B) phosphorus (mean ± 1SE, n = 5) of CPOM after the initial 14-day incubation period and prior to shredder feeding in nutrient-enriched and reference treatments. Percent (C) nitrogen and (D) phosphorus of FPOM produced (mean ± 1SE, n = 15) in treatments with shredders present following the 72-hour feeding period in nutrient-enriched and reference treatments. Letters indicate significant differences in treatments (ANOVA $p < 0.05$). Other designations as in Fig. 4.1.

Fig. 4.4. Relationship between fungal biomass associated with CPOM (A) N ($y = 0.0073x + 0.5778$) and (B) P content ($y = 0.0005x + 0.0255$) in treatments with fungi and without shredders. Only treatments without shredders were used to avoid potential selective feeding

effects (e.g., lower nutrient content on CPOM when shredders were present). Symbols represent individual microcosms.

Fig. 4.5. Relationship between CPOM-associated fungal biomass and CPOM mass loss in treatments with and without shredders. Nutrient-enriched trials are represented by 'NP' and ambient nutrient trials represent by 'Ref.' Symbols represent individual microcosms.

Fig. 4.1:

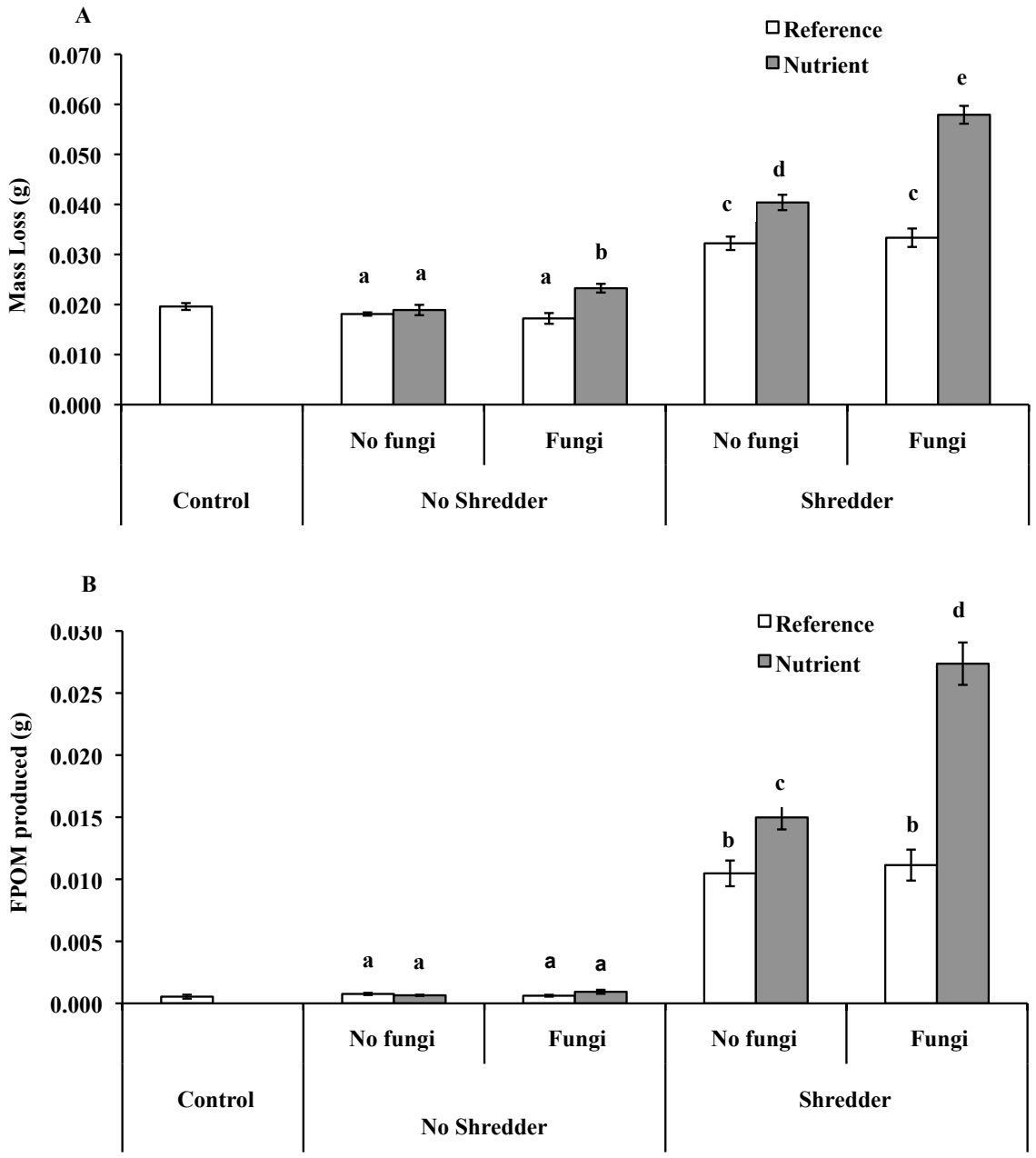

A
Reference
Nutrient
Mass Loss (g)
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
a
a
a
b
c
d
c
e
No fungi
Fungi
No fungi
Fungi
Control
No Shredder
Shredder
B
Reference
Nutrient
FPOM produced (g)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
a
a
a
a
b
c
b
d
No fungi
Fungi
No fungi
Fungi
Control
No Shredder
Shredder

Fig. 4.2:

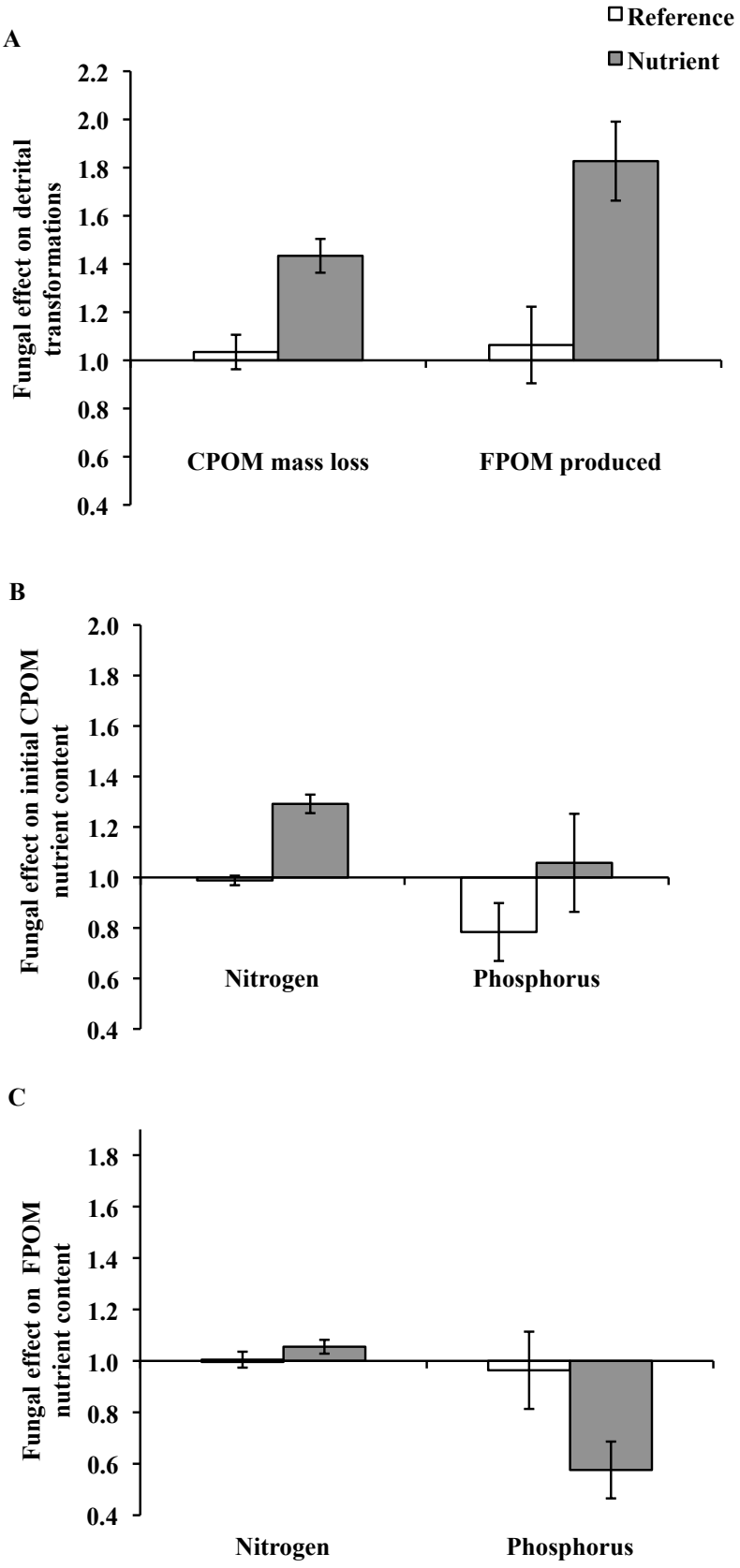

A
Reference
Nutrient
Fungal effect on detrital transformations
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
CPOM mass loss
FPOM produced
B
Fungal effect on initial CPOM nutrient content
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
Nitrogen
Phosphorus
C
Fungal effect on FPOM nutrient content
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
Nitrogen
Phosphorus

Fig. 4.3:

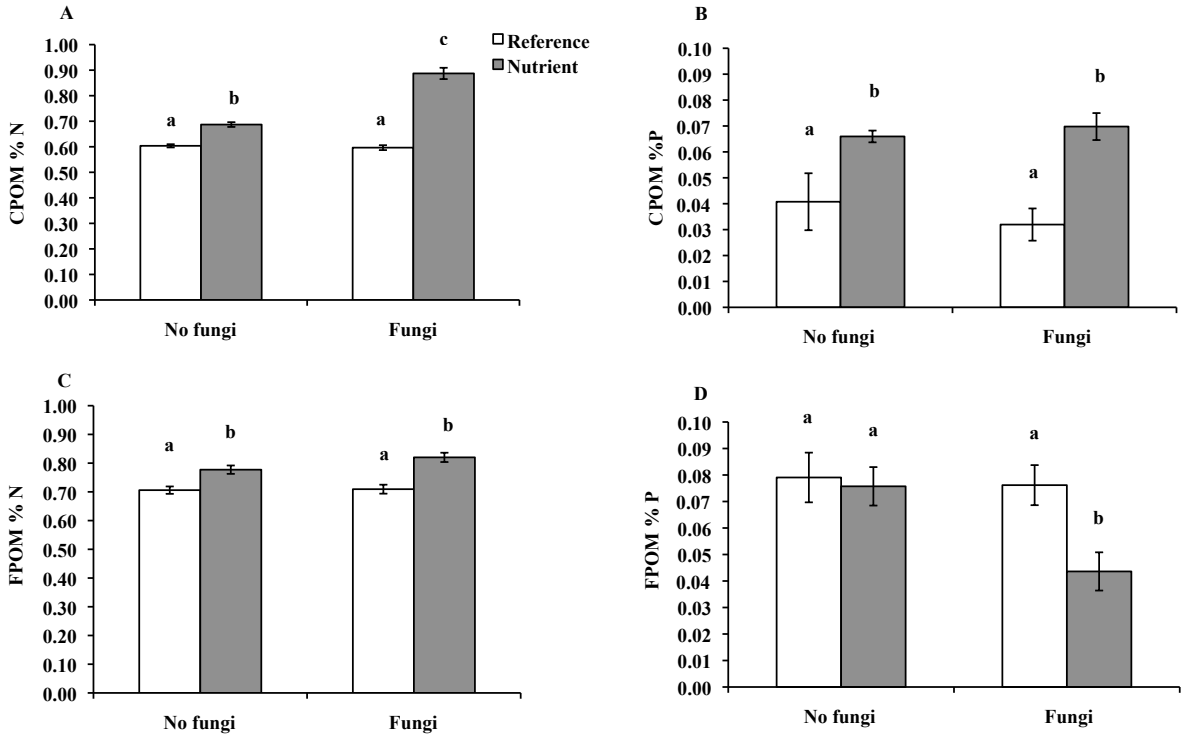

A
CPOM % N
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
a
b
a
c
Reference
Nutrient
No fungi
Fungi
B
CPOM %P
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
a
b
a
b
No fungi
Fungi
C
FPOM % N
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
a
b
a
b
No fungi
Fungi
D
FPOM % P
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
a
a
a
b
No fungi
Fungi

Fig. 4.4:

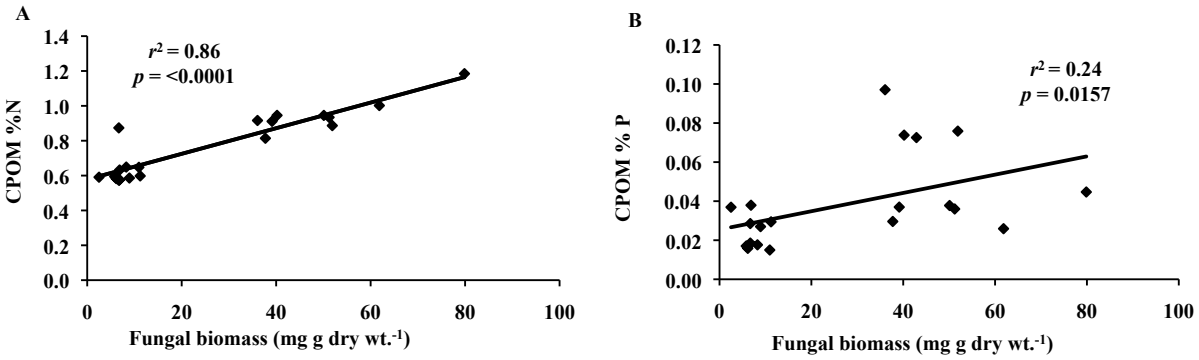
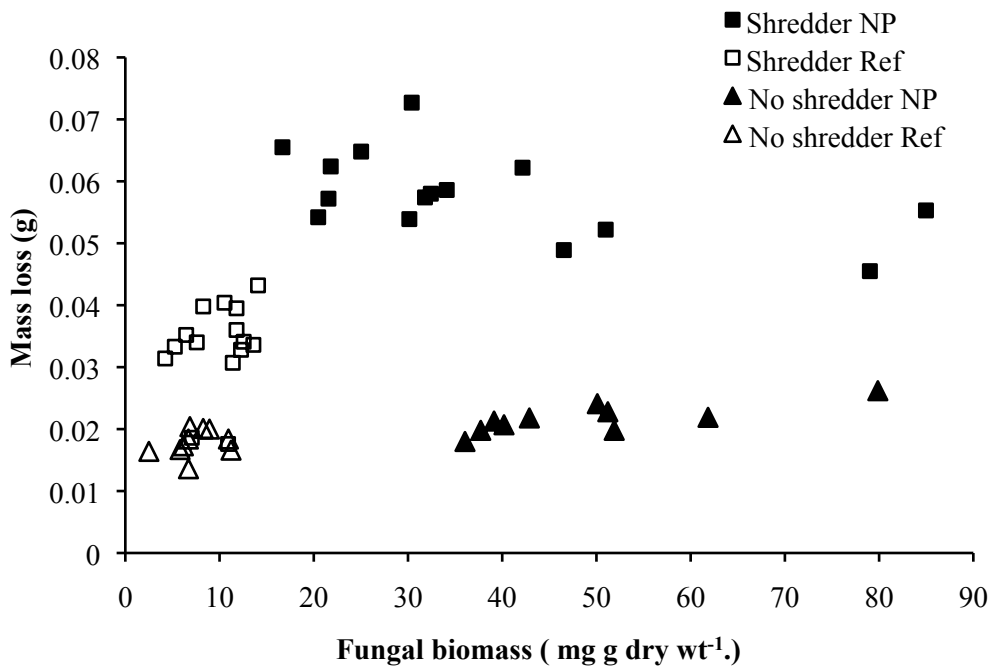

A
r^2 = 0.86
p = <0.0001
CPOM %N
Fungal biomass (mg g dry wt.$^{-1}$)
B
r^2 = 0.24
p = 0.0157
CPOM % P
Fungal biomass (mg g dry wt.$^{-1}$)

Fig 5:


Shredder NP
Shredder Ref
No shredder NP
No shredder Ref
Mass loss (g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
10
20
30
40
50
60
70
80
90
Fungal biomass (mg g dry wt-1.)

CHAPTER 5

CONCLUSIONS

Determining the response of a variety of ecosystem types to nutrient enrichment is necessary to manage and protect valuable natural resources. The objective of this dissertation was to examine the response of basal resources and their associated consumers to moderate levels of nutrient enrichment.

In addition to increased rates of leaf litter breakdown due to nutrient enrichment, Chapter 2 quantified the relative contribution of decomposer and detritivores to breakdown in our study streams. Leaf litter breakdown rates increased 4-5X under enriched conditions. Fungal biomass peaked at an earlier date and was higher in the treatment stream, while shredder biomass peaked either at the same time or shortly after peaks in fungal biomass. shredders dominated biological contributions to breakdown in the reference stream, though fungi contributed as much as shredders to breakdown in the treatment stream at similar stages of mass loss. Application of the Hieber-Gessner model to these streams suggests that nutrient enrichment may cause shifts toward increasing fungal and shredder contribution to leaf litter breakdown, intensifying losses of carbon through both respiration and downstream transport.

In Chapter 3, we observed a differential response to nutrients on CPOM vs. FBOM substrates. The response of CPOM (both maple and rhododendron substrates) was primarily positive, but the response of FBOM to enrichment was unexpectedly low. Nutrient enrichment increased fungal biomass and microbial respiration and decreased

C:N and C:P on CPOM substrates, suggesting a general increase in resource quality. Estimates of TERs and comparison to associated organic matter resources in each stream suggest that enrichment may reduce P limitation for shredders while potentially increasing C limitation for collectors. These results highlight potential mechanisms for food web shifts and pathways of carbon processing that may result from nutrient enrichment of detritus-based systems.

Chapter 4 examined the role of aquatic fungi in organic matter transformations in a laboratory experiment. CPOM mass loss and FPOM production were highest in treatments with both fungi and elevated nutrients. Both the presence of fungi and exogenous nutrients affected the N content of CPOM, but only nutrients had an effect on P content. Although fungi increased the availability of FPOM, the combined effects of fungi and nutrients did not appear to trickle down to FPOM nutrient content during organic matter transformations. These results suggest that aquatic fungi play a critical role in facilitating energy and nutrient flow through both CPOM and FPOM food web pathways and that their ability to mediate organic matter transformations are significantly influenced by nutrient enrichment.

Nutrient enrichment in detritus-based headwater streams has the potential to affect both instream communities as well as downstream ecosystems through export due to increased fragmentation of leaf material, production of fecal material by macroinvertebrates, and microbial maceration. The prevalence of detritus-based stream systems and the widespread nature of eutrophication make it imperative that we understand the mechanisms driving the response to nutrient enrichment in streams that are net heterotrophic and/or that occur via heterotrophic pathways.

APPENDIX A

Macroinvertebrate biomass (mg g AFDM^{-1}) associated with leaf packs in the reference and treatment streams on individual sampling dates. Taxa are grouped by functional feeding group (as defined by Wallace et al. 1999) and identified to genus or lowest possible taxonomic group. Days in stream indicates the number of days that leaf packs were allowed to incubate in each stream prior to removal and analysis. ND = no data due to lack of remaining leaf litter after d 49 in the treatment stream. NI = non-insect taxon.

			Days in Stream					
Functional group and taxon	**Order**	**Site**	**7**	**14**	**28**	**37**	**49**	**108**
Shredders								
Fattigia	Trichoptera	Reference	0.0182	0.0170	0.2540	0.0977	0.1444	0.1281
		Treatment	0.0003	0.0085	0	0.0033	0.0302	ND
Lepidostoma	Trichoptera	Reference	0.0146	0.0587	0.0995	0.2340	0.1834	0.0728
		Treatment	0.0080	0.0189	0.0532	0.0455	0.1969	ND
Leuctra	Plecoptera	Reference	0.0028	0.0139	0.0520	0.0456	0.1730	0.2144
		Treatment	0.0002	0.0055	0.0044	0.0062	0.0686	ND
Limonia	Diptera	Reference	0	0	0	0.0096	0.0232	0
		Treatment	0	0	0	0	0.0342	ND
Molophilus	Diptera	Reference	0.0049	0	0.0135	0	0.0157	0.0029
		Treatment	0	0	0	0	0	ND
Pycnopsyche	Trichoptera	Reference	0.0615	0.0314	0.1031	0.0704	0.0880	0.5813
		Treatment	0.2954	0.9930	1.8701	6.0902	32.7902	ND
Tallaperla	Plecoptera	Reference	0.2019	0.7069	0	0.3839	0.6534	0.1028
		Treatment	0	0.4188	1.3342	0.6667	4.1865	ND
Tipula	Diptera	Reference	0	0	0	0	0.5570	0.0766
		Treatment	0	0	0	0	0.0304	ND

			Days in Stream					
Functional group and taxon	**Order**	**Site**	**7**	**14**	**28**	**37**	**49**	**108**
Scrapers								
Baetis	Ephemeroptera	Reference	0	0	0	0	0	0
		Treatment	0	0	0	0	0.0678	ND
Elmidae	Coleoptera	Reference	0	0	0	0	0	0.1881
		Treatment	0	0	0	0	0	ND
Predators								
Acari	NI	Reference	0.0009	0.0021	0.0052	0.0044	0.0059	0.0135
		Treatment	0.0006	0.0011	0.0037	0.0013	0.0005	ND
Beloneuria	Plecoptera	Reference	0.1405	0.6098	0.0358	0.9288	0.8244	0.6587
		Treatment	0.1783	0.6035	1.6829	1.7623	3.8628	ND
Ceratopogonidae	Diptera	Reference	0.0020	0.0075	0.1188	0.1153	0.1090	0.3302
		Treatment	0.0107	0	0	0.0575	0.4536	ND
Tanypodinae	Diptera	Reference	0.0016	0.0025	0.0422	0.0177	0.0212	0.4204
		Treatment	0.0083	0.0061	0.0088	0	0.0458	ND
Cordulegaster	Odonata	Reference	0	0	0	0	0	0.0606
		Treatment	0.0252	0	0.1357	0	0	ND
Dicranota	Diptera	Reference	0.0016	0.0036	0.0229	0.0046	0.0124	0.0220
		Treatment	0.0006	0.0021	0.0072	0.0089	0.0955	ND
Empididae	Diptera	Reference	0	0	0.0042	0	0	0
		Treatment	0	0	0	0.0007	0	ND
Hexatoma	Diptera	Reference	0	0	0.2237	0.0025	0.0099	0.1864
		Treatment	0	0	0	0.0046	0.0749	ND
Isoperla	Plecoptera	Reference	0.0188	0.0154	0.0332	0.0164	0.0335	0.0824
		Treatment	0.0464	0.0873	0.3652	1.4365	0.2930	ND

			Days in Stream					
Functional group and taxon	**Order**	**Site**	**7**	**14**	**28**	**37**	**49**	**108**
Lanthus	Odonata	Reference	0	0.2788	0	0.0924	0.7591	1.1970
		Treatment	0	0.0355	0	0.1130	0.1420	ND
Pilaria	Diptera	Reference	0	0	0	0	0.0390	0
		Treatment	0	0	0	0	0	ND
Polycentropus	Trichoptera	Reference	0	0	0.0152	0	0	0
		Treatment	0	0	0.0234	0	0	ND
Pseudolimnophora	Diptera	Reference	0.0005	0	0.0151	0.0276	0.0238	0.0764
		Treatment	0	0	0	0.0507	0.0088	ND
Rhyacophila	Trichoptera	Reference	0.0093	0.0031	0	0.0191	0.1866	0.1480
		Treatment	0	0.0200	0.5480	0.1771	0.8385	ND
Sweltsa	Plecoptera	Reference	0	0	0	0	0	0
		Treatment	0.0508	0.0597	0	0	0.2031	ND
Turbellaria	NI	Reference	0.0024	0.0211	0.0911	0.1038	0.0836	0.0092
		Treatment	0	0.0031	0.1241	0.0719	0.0548	ND
Pedicia	Diptera	Reference	0	0	0	0.0041	0.0021	0
		Treatment	0	0	0.0015	0	0.0105	ND
Collector-gatherers								
Amphinemura	Plecoptera	Reference	0.0212	0.0081	0.0142	0.0144	0.0401	0.0070
		Treatment	0.0425	0.0497	0.2009	0.2431	0.6647	ND
Chironomidae (non-Tanypodinae)	Diptera	Reference	0.0512	0.0597	0.4681	0.2695	0.4416	5.8019
		Treatment	0.1712	0.1574	0.3312	0.4436	1.9127	ND
Collembola	NI	Reference	0.0109	0.0082	0.0217	0.0138	0.0150	0.0063
		Treatment	0.0180	0.0422	0.0749	0.0660	0.0426	ND

			Days in Stream					
Functional group and taxon	**Order**	**Site**	**7**	**14**	**28**	**37**	**49**	**108**
Copepod	NI	Reference	0.0011	0.0065	0.0956	0.0562	0.0635	0.1020
		Treatment	0.0082	0.0098	0.0335	0.1105	0.4324	ND
Dixa	Diptera	Reference	0.0655	0.0321	0.0193	0.0150	0.0065	0.0058
		Treatment	0.0308	0.0333	0.1019	0.0780	0.0684	ND
Leptotarsus	Diptera	Reference	0	0	0.0037	0.0683	0	0
		Treatment	0	0	0	0	0	ND
Nematoda	NI	Reference	0	0	0.0020	0.0000	0.0002	0.0038
		Treatment	0	0	0.0004	0.0001	0	ND
Nymphomyiidae	Diptera	Reference	0.0003	0.0003	0	0.0010	0.0002	0
		Treatment	0.0005	0	0	0	0	ND
Oligochaeta	NI	Reference	0.0065	0.0019	0.0251	0.0288	0.1098	0.0934
		Treatment	0.0313	0.0104	0.0105	0.0844	0.1012	ND
Paraleptophlebia	Ephemeroptera	Reference	0	0	0.0314	0.0062	0	0.0267
		Treatment	0.0982	0.0783	0.5223	0.2539	0.4995	ND
Parapsyche	Trichoptera	Reference	0	0	0	0	0.0741	0
		Treatment	0	0	0.0597	0	0.0353	ND
Seratella	Ephemeroptera	Reference	0.0392	0.0057	0	0	0	0
		Treatment	0	0.0051	0	0.0327	0.0102	ND
Stenonema	Ephemeroptera	Reference	0	0	0.0936	0.1261	0	0
		Treatment	0.2334	0.6730	0.3915	0.2237	0.1040	ND
Wormaldia	Trichoptera	Reference	0	0.0048	0.4581	0.0149	0.0200	0.0329
		Treatment	0.0033	0.0026	0.7350	0.0740	0.1243	ND
Ostracoda	NI	Reference	0.0002	0	0.0068	0.0029	0.2134	0.0064
		Treatment	0.0002	0.0001	0.0013	0.0010	0.0004	ND

			Days in Stream					
Functional group and taxon	**Order**	**Site**	**7**	**14**	**28**	**37**	**49**	**108**
Collector-filterers								
Diplectrona	Trichoptera	Reference	0.0929	0	0.1001	0.0183	0.2905	0.2485
		Treatment	0.0619	0.1260	0.5711	1.0460	1.7180	ND

APPENDIX B

Microbial biomass and activity and substrate nutrient content plotted over time in the reference (Ref) and treatment (NP) streams.

Fig. 1. Fungal biomass (mg C mg $AFDM^{-1}$) over time in the reference and treatment streams on a) CPOM and b) FBOM. Time is shown as days in stream for CPOM and sample date for FBOM. Error bars indicate ± 1 SE.

Fig. 2. Bacterial biomass (mg C mg $AFDM^{-1}$) over time in the reference and treatment streams on a) CPOM and b) FBOM. Format as described in Fig. 1.

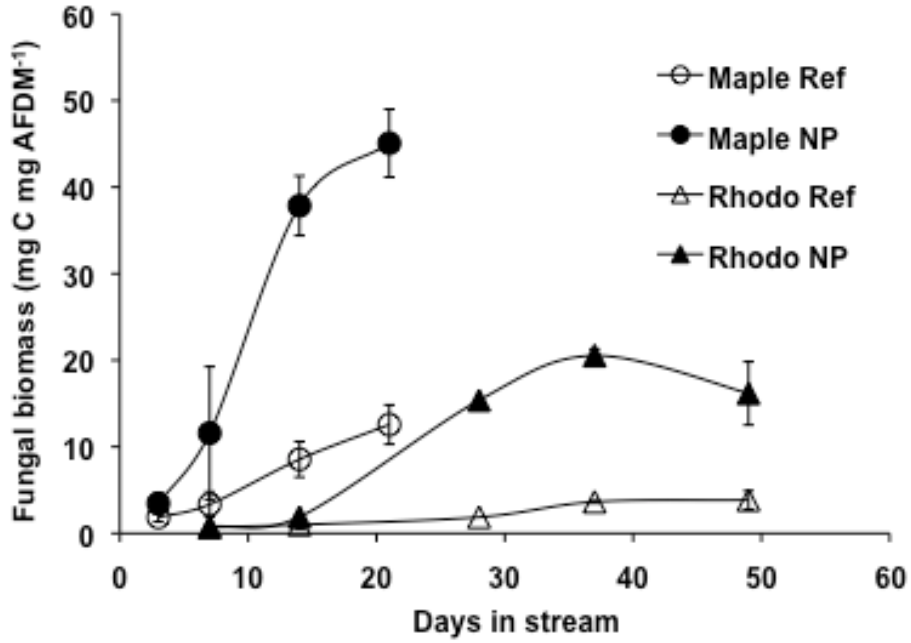
Fig. 3. Microbial respiration (mg O_2 g leaf $AFDM^{-1}$ hr^{-1}) over time in the reference and treatment streams on a) CPOM and b) FBOM. Format as described in Fig. 1.

Fig. 4. Substrate C:N ratio over time in the reference and treatment streams on a) CPOM and b) FBOM. CPOM initial values (day 0) shown for reference but excluded from ANOVAs. Format as described in Fig. 1.

Fig. 5. Substrate C:P ratio over time in the reference and treatment streams on a) CPOM and b) FBOM. CPOM initial values (day 0) shown for reference but excluded from ANOVAs. Format as described in Fig. 1.

Fig. 1.

a)


Fungal biomass (mg C mg AFDM-1)
Days in stream
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP
0
10
20
30
40
50
60

b)

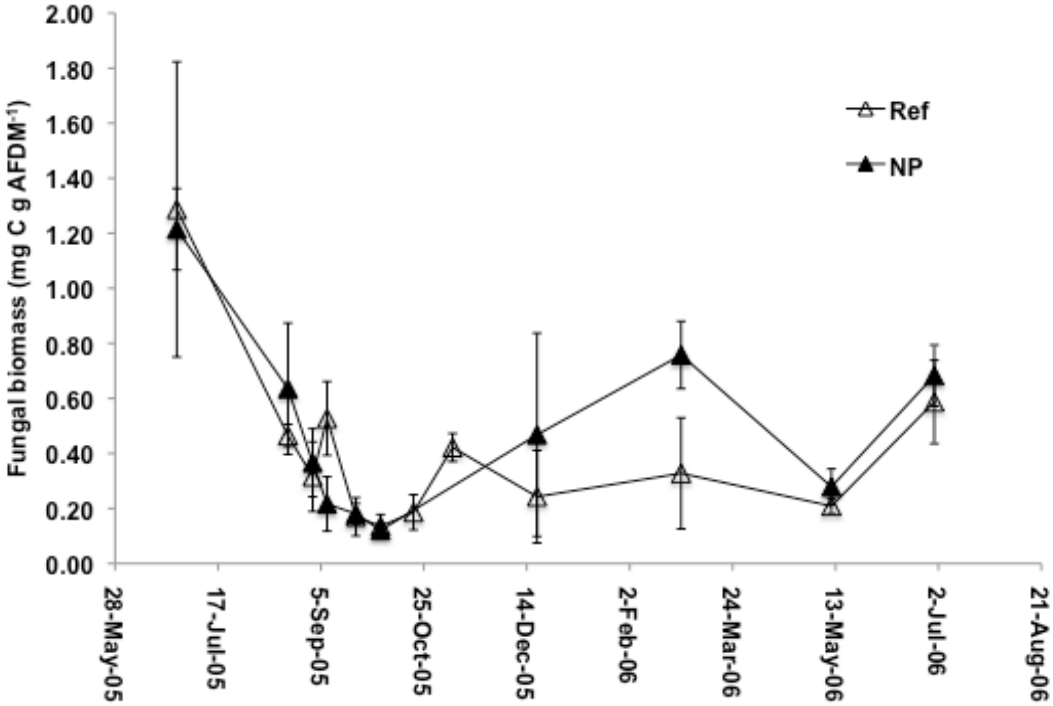
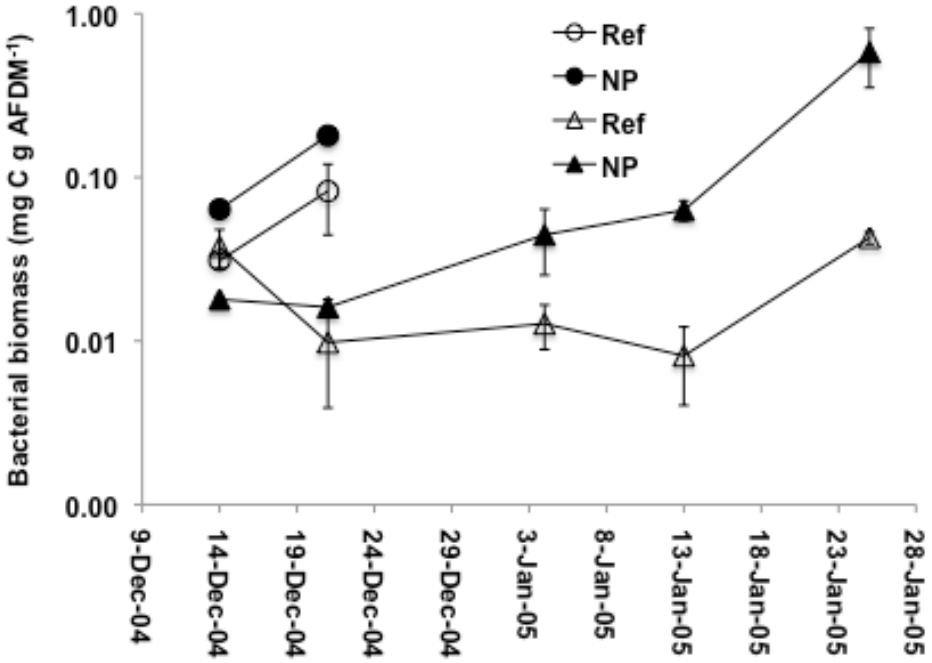

Fungal biomass (mg C g AFDM-1)
Ref
NP
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
28-May-05
17-Jul-05
5-Sep-05
25-Oct-05
14-Dec-05
2-Feb-06
24-Mar-06
13-May-06
2-Jul-06
21-Aug-06

Fig. 2.

a)


Bacterial biomass (mg C g AFDM-1)
1.00
0.10
0.01
0.00
Ref
NP
Ref
NP
9-Dec-04
14-Dec-04
19-Dec-04
24-Dec-04
29-Dec-04
3-Jan-05
8-Jan-05
13-Jan-05
18-Jan-05
23-Jan-05
28-Jan-05

b)

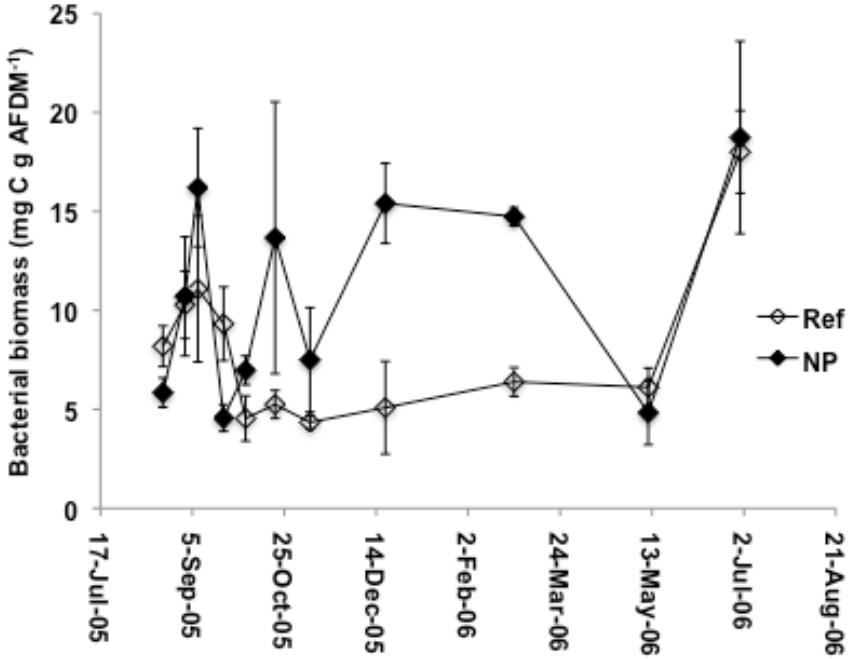

Bacterial biomass (mg C g AFDM-1)
25
20
15
10
5
0
Ref
NP
17-Jul-05
5-Sep-05
25-Oct-05
14-Dec-05
2-Feb-06
24-Mar-06
13-May-06
2-Jul-06
21-Aug-06

Fig. 3.

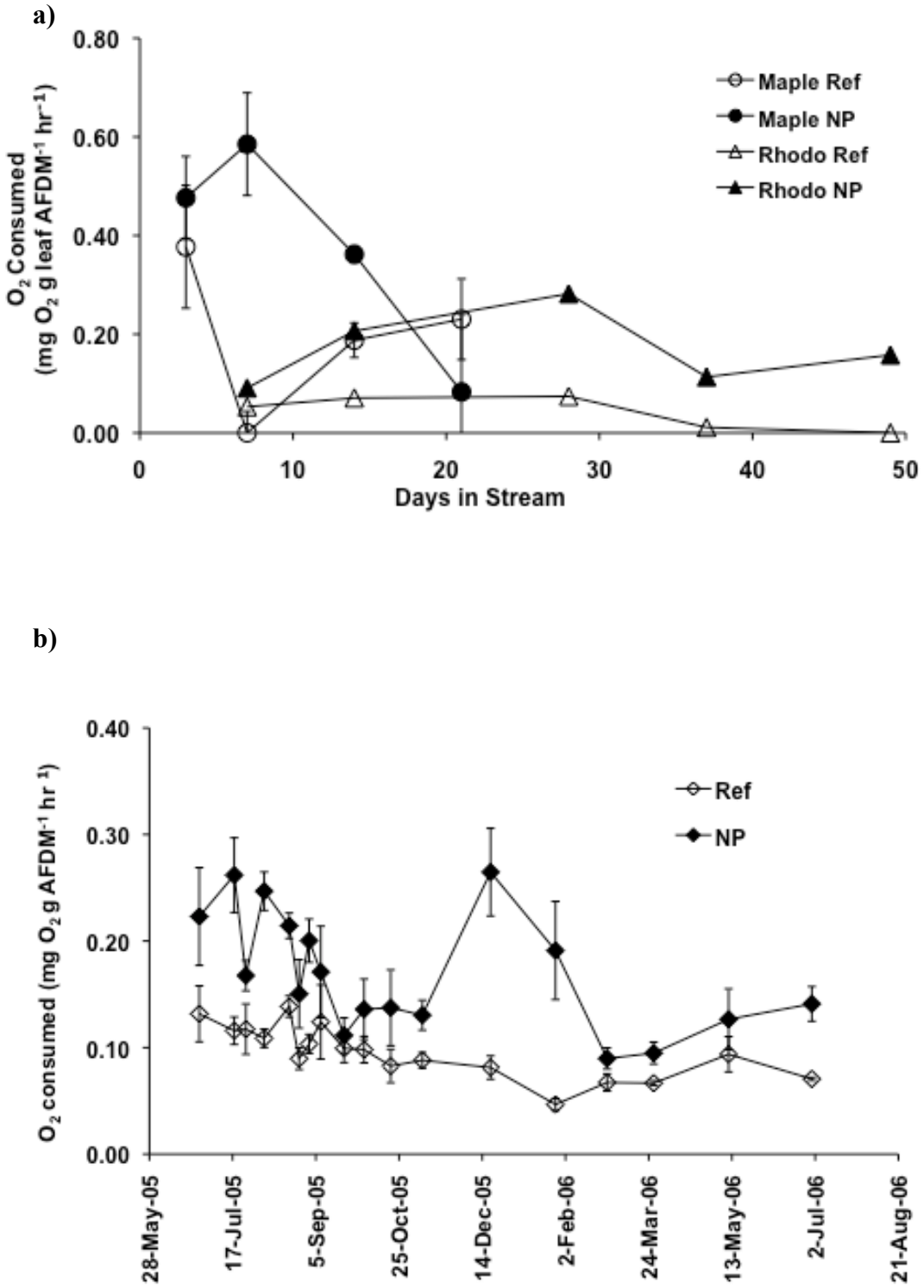
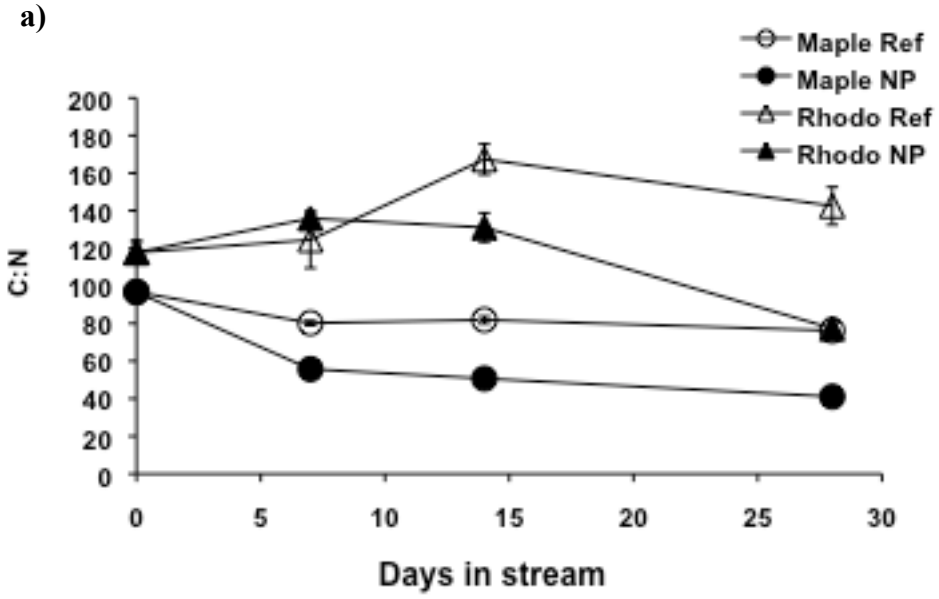

a)
0.80
0.60
0.40
0.20
0.00
O_2 Consumed
(mg O_2 g leaf AFDM^{-1} hr^{-1})
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP
0
10
20
30
40
50
Days in Stream
b)
0.40
0.30
0.20
0.10
0.00
O_2 consumed (mg O_2 g AFDM^{-1} hr ^{1})
Ref
NP
28-May-05
17-Jul-05
5-Sep-05
25-Oct-05
14-Dec-05
2-Feb-06
24-Mar-06
13-May-06
2-Jul-06
21-Aug-06

Fig. 4.

a)


Maple Ref
Maple NP
Rhodo Ref
Rhodo NP
C:N
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Days in stream

b)

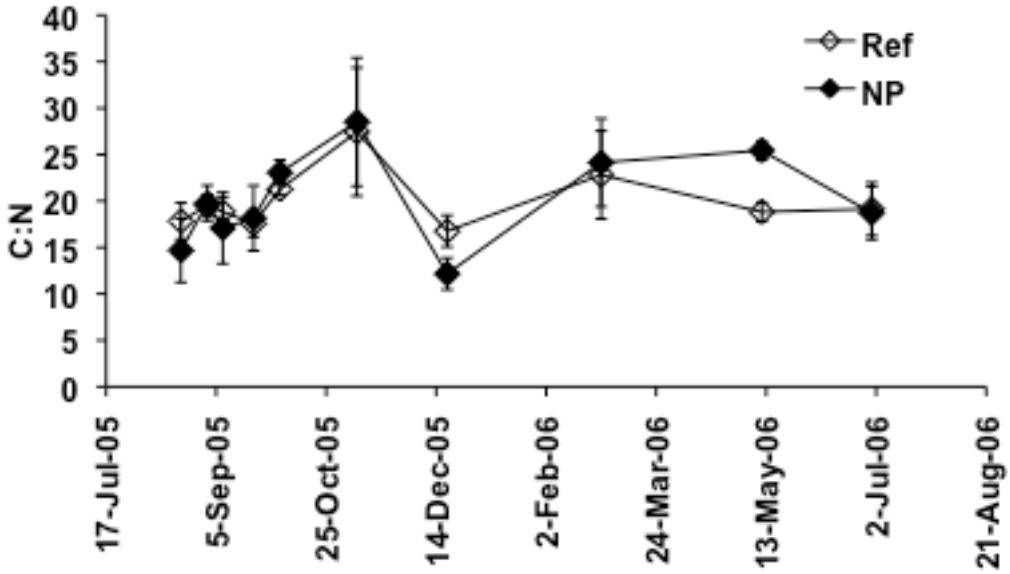
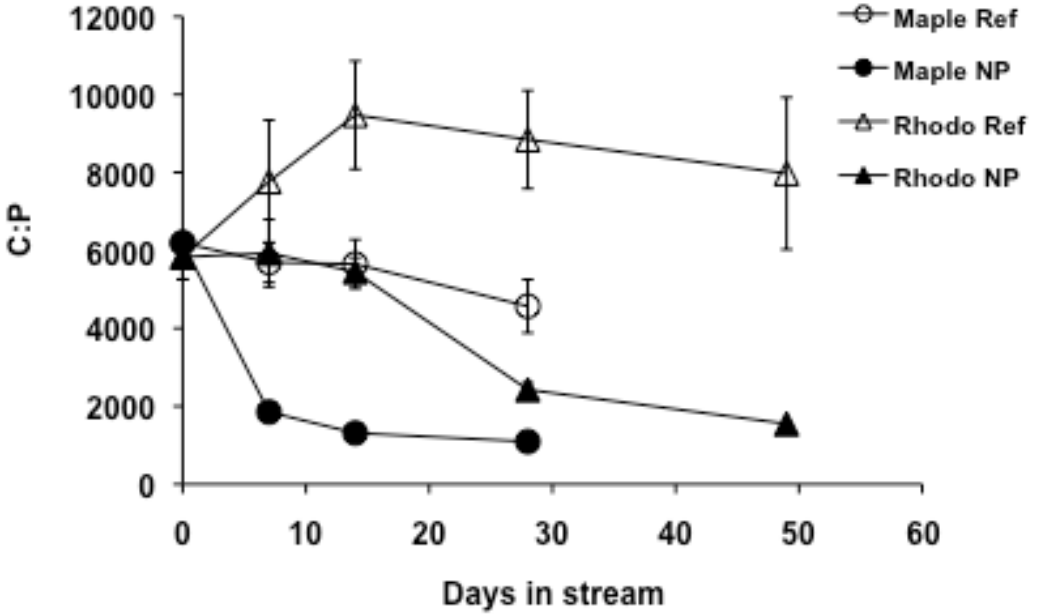
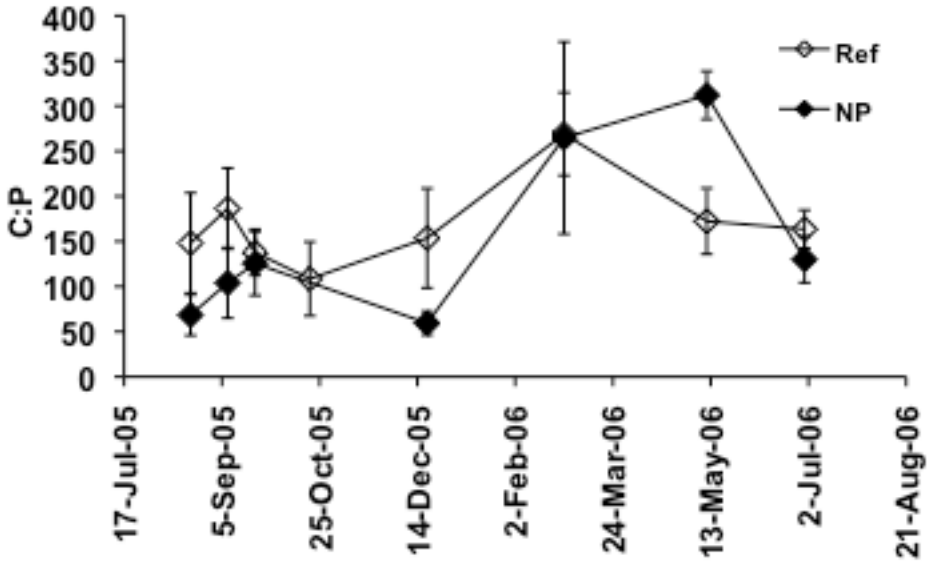

Ref
NP
C:N
40
35
30
25
20
15
10
5
0
17-Jul-05
5-Sep-05
25-Oct-05
14-Dec-05
2-Feb-06
24-Mar-06
13-May-06
2-Jul-06
21-Aug-06

Fig. 5.


12000
10000
8000
6000
4000
2000
0
C:P
0
10
20
30
40
50
60
Days in stream
Maple Ref
Maple NP
Rhodo Ref
Rhodo NP

b)


400
350
300
250
200
150
100
50
0
C:P
17-Jul-05
5-Sep-05
25-Oct-05
14-Dec-05
2-Feb-06
24-Mar-06
13-May-06
2-Jul-06
21-Aug-06
Ref
NP