THE RESPONSE OF DETRITAL AND AUTOTROPHIC RESOURCES TO LONG-TERM NUTRIENT ENRICHMENT IN A DETRITUS-BASED HEADWATER STREAM

by

JENNIFER LYNN GREENWOOD

(Under the Direction of Amy D. Rosemond)

ABSTRACT

Enrichment of aquatic ecosystems with nitrogen and phosphorus is one of the most significant anthropogenic impacts to surface waters worldwide. Relatively little is known about nutrient effects on detritus-based stream systems relative to autotroph-based systems. This study examined the effects of a long-term nitrogen and phosphorus enrichment on autotrophic and heterotrophic portions of the resource base of a forested detritus-based headwater stream at the Coweeta Hydrologic Laboratory in the southern Appalachian mountains. I assessed the affects of 2 years of nutrient enrichment on the detrital and autotrophic resources: leaf litter, periphyton and bryophytes. Nutrient enrichment strongly affected processing rates of allochthonous leaf inputs. Leaf breakdown rates, microbial activity on leaf material, the nitrogen content of leaf litter, and invertebrate biomass associated with leaf litter, all increased with nutrient enrichment. Increased processing rates also accelerated the flux of nitrogen to invertebrate biomass from leaf litter standing crop. Overall, the effect of enrichment was slightly stronger on a lower quality (lower nitrogen content) leaf, rhododendron, relative to a higher quality leaf type, red maple, suggesting nutrient enrichment may not affect all detrital resources equally. The response of autotrophs to nutrient enrichment was less dramatic, primarily due to strong light limitation.

Algal biomass measured as chlorophyll *a* and algal growth rates increased with nutrient enrichment, with strongest effects in the early spring, when light levels reaching the stream were highest. The diatom-dominated algal species assemblages were not altered by nutrient enrichment and were more related to seasonal effects. Bryophyte biomass also did not change with nutrient enrichment, potentially due to light limitation. Biomass of algal epiphytes on bryophytes, measured as biovolume, showed variable response to nutrient enrichment, and algal community patterns were more affected by substrate type (moss, liverwort or bedrock) than nutrient or light availability. Overall, results from this study show that nutrient enrichment had strong effects on primarily the detrital resources in a headwater stream, with subtle effects on the autotrophic resources. Thus, algal-based measures of nutrient impacts currently used for running waters may be inappropriate for detritus-based stream ecosystems.

INDEX WORDS: Coweeta, nutrient enrichment, headwater stream, detritus, leaf breakdown, invertebrate, periphyton, aquatic bryophyte, algal epiphyte

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Nutrient loading of nitrogen and phosphorus is an important problem in aquatic ecosystems (Carpenter et al. 1998). The demands of modern civilization and an exponentially increasing human population are causing perturbations of biogeochemical cycles and inputs of nutrients to aquatic systems at unprecedented rates. For example, the amount of nitrogen fixed by industry is at least equal to natural sources (Vitousek et al. 1997; Vitousek et al. 1997), and human activity explains most of the global variation in nitrate export in world rivers (Caraco and Cole 1999). Also, mining of phosphorus has enhanced loading to aquatic systems (Caraco 1993), and increased phosphorus storage in terrestrial habitats provides a continuing future threat of phosphorus enrichment due to runoff (Bennett et al. 2001).

Typical concerns with nutrient enrichment generally deal with the autotrophic response since the negative effects of enrichment are usually most obvious in the form of algal blooms or excessive plant growth (Carpenter et al. 1998). This excessive growth of autotrophs can cause problems such as interference with the use of water for recreation, irrigation or drinking water, decreased oxygen availability in the water column potentially resulting in fish kills (Carpenter et al. 1998), and toxic algal blooms presenting health hazards to the public or livestock (Paerl 1997). Thus, much work on how aquatic systems respond to nutrient enrichment have focused on lake and stream systems that are autotroph-based, where algal plankton or periphyton make up a large portion of the resource base (Schindler et al. 1973; Peterson et al. 1985; Peterson et al. 1993).

In spite of the focus on autotroph-based systems, many aquatic ecosystems are detritus-based, where the majority of the food web depends on allochthonous detritus as the dominant energy source. The importance of detritus has long been recognized for low-order forested streams where it plays a dominant role in production and ecosystem function (Fisher and Likens 1973; Vannote et al. 1980; Wallace et al. 1999; Moore et al. 2004). Headwater streams also play an important role in water quality and in the health of downstream ecosystems (Meyer and Wallace 2001). Headwater streams frequently retain and transform more than 50% of nitrogen inputs, preventing downstream nutrient loading (Peterson et al. 2001), and also perform a large portion of organic matter processing for the entire watershed (Meyer and Wallace 2001). In spite of the importance of detritus-based ecosystems, we know much less about how nutrient enrichment affects detrital pathways relative to autotrophic pathways in aquatic food webs.

The effects of nutrients on detritus and detritus-based food webs in streams have only recently begun to be studied (Suberkropp and Chauvet 1995; Rosemond et al. 2001), and some general trends have become evident. Nutrient enrichment often results in positive effects on leaf litter-associated microbial biomass and productivity (Suberkropp and Chauvet 1995; Weyers and Suberkropp 1996). The stimulation of the microbial community has been shown to affect higher trophic levels that feed on leaf litter. For example, nutrient enrichment of stream water has resulted in increased abundance of collectors (Pearson and Connoly 2000; Rosemond et al. 2001) and shredding stoneflies (Robinson and Gessner 2000). As a result of increased activity of microbes and/or invertebrates, many studies have shown leaf breakdown rates to increase with increased levels of stream water nutrients, changing the timing of availability of allochthonous leaf material for consumers (Kaushik and Hynes 1971; Howarth and Fisher 1976; Elwood et al. 1981; Meyer and Johnson 1983; Suberkropp and Chauvet 1995; Weyers and Suberkropp 1996;

Robinson and Gessner 2000; Grattan II and Suberkropp 2001; Rosemond et al. 2002). In contrast to autotrophs which generally increase the amount of carbon in the resource base, detrital carbon tends to decrease with nutrient enrichment.

The response of autotrophs to nutrient enrichment in detritus-based streams has been studied much less compared to their response in autotroph-based systems. The response of autotrophs, primarily algae and bryophytes, to nutrient enrichment in forested headwater streams is typically constrained by other factors. Short-term experimental work in forested headwater streams indicates that the response of algal biomass to nutrient enrichment is limited by ambient light levels (Gregory 1980; Triska et al. 1983; Lowe et al. 1986; Hill and Knight 1988; Winterbourn 1990; Rosemond 1993; Wellnitz et al. 1996; Rosemond et al. 2000; Hill et al. 2001; Bernhardt and Likens 2004). Thus, it is reasonable to assume that the response of periphyton to nutrient enrichment in a forested headwater stream may be minimal due to light limitation. Bryophyte biomass has also been shown to increase in response to nutrient enrichment in well-lit streams (Bowden et al. 1994; Slavik et al. 2004), however, in a shaded, forested stream bryophyte biomass showed no response to enrichment (Steinman 1994). Thus, bryophytes may also be limited by light more so than nutrients in forested streams. However, most studies of light and/or nutrient limitation on autotrophs are short term, and longer term studies may be necessary to determine the ultimate effects of nutrients on autotrophs in heavily shaded systems.

Dissertation Overview

Work for this dissertation was conducted at the Coweeta Hydrologic Laboratory (CHL), a USDA Forest Service research facility, located in the Blue Ridge Mountain physiographic province in the southern Appalachian mountains in western North Carolina, USA. The headwater stream from Catchment 54 (C 54) at the CHL was continuously enriched with nitrogen and

phosphorus for two years beginning on 11 July 2000. The stream from Catchment 53 (C 53) served as the project reference stream. Pretreatment data were collected from both streams for at least one year prior to enrichment.

The purpose of this dissertation was to examine the response of both detrital and autotrophic components of the resource base to long-term nutrient enrichment. My research questions were:

(1) How will leaf litter of different quality and the associated invertebrates respond to long-term nutrient enrichment in a detritus-based headwater streams (Chapter 2)? Understanding how leaf litter will respond to nutrient enrichment is critical to understanding the response of the rest of the food web, since allochthonous leaf litter is the primary source of energy for the food web in these streams (Wallace et al. 1999). I measured breakdown rates, microbial respiration rates, leaf C:N ratios, invertebrate communities and the amount of nitrogen contained in leaf material vs. invertebrate biomass in litter bags of a low quality leaf, rhododendron (*Rhododendron maximum*), and a higher quality leaf, red maple (*Acer rubrum*). I predicted that nutrient enrichment would stimulate breakdown rates, microbial activity measured as respiration, decrease C:N content of leaf material, increase invertebrate abundance and biomass and increase the relative amount of nitrogen contained in invertebrate biomass vs. leaf material. I also predicted that rhododendron would show more of an increase in breakdown rates and nutrient content due to nutrient enrichment relative to red maple leaves.

(2) How will epilithic periphyton respond to nutrient enrichment (Chapter 3)? Light is very low in these forested headwater streams, and, potentially, algal biomass may not respond at all to nutrient enrichment. However, differential water chemistry may result in shifts in algal species assemblages. I measured algal biomass as ash-free dry mass and chlorophyll *a*,

determined algal species composition, and assessed algal growth rates as a proxy measure of productivity. I hypothesized that due to light limitation, nutrient enrichment would have little overall effect on algal biomass, and cellular growth rates of periphyton would be constrained. However, because of variation in nutrient optima among algal taxa, I predicted that periphyton assemblage structure would be altered with changes in the relative abundance of common species.

(3) Will periphyton biomass and algal species assemblages associated with bryophytes differ in response to nutrient enrichment and light availability relative to periphyton from bedrock (Chapter 4)? Periphyton community response to environmental manipulation is frequently assessed from epilithic communities (Borchardt 1996). However, bryophytes can be an important periphyton substratum in many headwater streams (Naiman 1983; Slack and Glime 1985; Glime and Vitt 1987; Steinman and Boston 1993; Suren 1993; Cattaneo and Fortin 2000). Further, bryophytes can potentially support a distinct algal flora compared to epilithic substrata, as different algal communities are often found on macrophytes compared to rock (Pentecost 1991; Rothfritz et al. 1997; Passy et al. 1999; Lim et al. 2001). The potential differential response to nutrient enrichment by periphyton from bryophytes and epilithon and also the potential role played by nutrient and light availability in structuring epiphytic algal assemblages were assessed. I measured nutrient effects on bryophyte biomass and the biovolume and community structure of associated algal epiphytes in the enriched and reference streams during periods of low and high light availability. I predicted that bryophyte biomass would increase with nutrient enrichment, thus increasing the amount of substrata available for epiphyte colonization. Also, bryophytes would support more periphyton biomass vs. bedrock per area of streambed, and biomass of periphyton would increase on all substrata with nutrient enrichment.

Further, I predicted that periphytic species assemblages would differ among moss, liverwort and bedrock substrata, and the impact of nutrient enrichment on periphytic species assemblages would depend on substratum type and be most pronounced during seasonal high light availability.

How nutrient enrichment can affect detritus-based aquatic ecosystems such as headwater streams is an important component of aquatic ecology that has not been adequately examined. Predictions of nutrient enrichment effects in aquatic ecosystems are typically based on models of how autotroph-dominated systems respond to enrichment and may not be appropriate in detritus-based systems. In autotroph-based systems, primary production tends to increase with enrichment, increasing the amount of carbon available to or flowing through the food web. Yet, nutrients may illicit a fundamentally different response from detritus, where available detrital carbon may ultimately decrease in quantity yet increase in quality. This dissertation will contribute to our understanding of how chronic nutrient enrichment controls resource quality and availability in detritus-based stream ecosystems, and will also enhance our ability to predict effects of nutrient enrichment on detrital food webs and ecosystem functioning.

References

Bennett, E. M., S. R. Carpenter and N. F. Caraco. 2001. Human impact on erodable phosphorus and eutrophication: a global perspective. BioScience 51: 227-234.

Bernhardt, E. S. and G. E. Likens. 2004. Controls on periphyton biomass in heterotrophic streams. Freshwater Biology 49: 14-27.

Borchardt, M. A. 1996. Nutrients. *In* Algal ecology: freshwater benthic ecosystems. R. J. Stevenson, M. L. Bothwell and R. L. Lowe., eds. San Diego, Academic Press pp. 183-227.

Bowden, W. B., J. C. Finlay and P. E. Maloney. 1994. Long-term effects of PO_4 fertilization on the distribution of bryophytes in an arctic river. Freshwater Biology 32: 445-454.

Caraco, N. F. 1993. Disturbance of the Phosphorus Cycle: A Case of Indirect Effects of Human Activity. Trends in Ecology and Evolution 8: 51-54.

Caraco, N. F. and J. J. Cole. 1999. Human impact on nitrate export: an analysis using major world rivers. Ambio 28: 167-170.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley and V. H. Smith. 1998. Nonpoint polllution of surface waters with phosphorus and nitrogen. Ecological Applications 8: 559-568.

Cattaneo, A. and L. Fortin. 2000. Moss distribution in streams of the Quebec Laurentian Mountains. Canadian Journal of Botany 78: 748-752.

Elwood, J. W., J. D. Newbold and A. F. Trimble. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146-158.

Fisher, S. G. and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs 43: 421-439.

Glime, J. M. and D. H. Vitt. 1987. A comparison of bryophyte species diversity and niche structure of montane streams and stream banks. Canadian Journal of Botany 65: 1824-1837.

Grattan II, R. M. and K. Suberkropp. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. Journal of the North American Benthological Society 20: 33-43.

Gregory, S. V. 1980. Effects of Light, Nutrients, and Grazing on Periphyton Communities in Streams. Dissertation. Oregon State University

Hill, W. R. and A. W. Knight. 1988. Nutrient and light limitation of algae in two northern California streams. Journal of Phycology 24: 125-132.

Hill, W. R., P. J. Mulholland and E. R. Marzolf. 2001. Stream ecosystem responses to forest leaf emergence in spring. Ecology 82: 2306-2319.

Howarth, R. W. and S. G. Fisher. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. Freshwater Biology 6: 221-228.

Kaushik, N. K. and H. B. N. Hynes. 1971. The fate of dead leaves that fall into streams. Archiv für Hydrobiologie 68: 465-515.

Lim, D. S. S., C. Kwan and M. S. V. Douglas. 2001. Periphyton diatom assemblages from Bathurst Island, Nanavut, Canadian High Arctic: an examination of community relationships and habitat preferences. Journal of Phycology 37: 379-392.

Lowe, R. L., S. W. Golladay and J. R. Webster. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. Journal of the North American Benthological Society 5: 221-229.

Meyer, J. L. and C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. Freshwater Biology 13: 177-183.

Meyer, J. L. and J. B. Wallace 2001. Lost Linkages and Lotic Ecology: Rediscovering Small Streams. *In* Ecology: Achievement and Challenge. N. J. Huntly and S. Levin. Oxford, eds. Blackwell Science. pp. 295-317.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7: 584-600.

Naiman, R. J. 1983. The annual pattern and spatial distribution of aquatic oxygen metabolism in boreal forest watersheds. Ecological Monographs 53: 73-94.

Paerl, H. W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other sources. Limnology and Oceanography 42: 1154-1165.

Passy, S. I., Y. Pan and R. L. Lowe. 1999. Ecology of the major periphytic diatom communities from the Mesta River, Bulgaria. International Review of Hydrobiology 84: 129-174.

Pearson, R. G. and N. M. Connoly. 2000. Nutrient enhancement, food quality and community dynamics in a tropical rainforest stream. Freshwater Biology 43: 31-42.

Pentecost, A. 1991. Algal and bryophyte flora of a Yorkshire (U.K.) hill stream: a comparative approach using biovolume estimations. Archiv für Hydrobiologie 121: 181-201.

Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. McKinely, M. C. Miller, J. R. Vestal, R. Ventullo and G. Volk. 1993. Biological responses of a tundra river to fertilization. Ecology 74: 653-672.

Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Lock, T. E. Ford, J. R. Vestal, V. L. McKinley, M. A. Hullar, M. C. Miller, R. M. Ventullo and G. S. Volk. 1985. Transformation of a Tundra River from Heterotrophy to Autotrophy by Addition of Phosphorus. Science 229: 1383-1386.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. V. Gregory and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292: 86-90.

Robinson, C. T. and M. O. Gessner. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia 122: 258-263.

Rosemond, A. D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia 94: 585-594.

Rosemond, A. D., P. J. Mulholland and S. H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Canadian Journal of Fisheries and Aquatic Sciences 57: 66-75.

Rosemond, A. D., C. M. Pringle, A. Ramirez and M. J. Paul. 2001. A test of top-down and bottom-up control in a detritus-based food web. Ecology 82: 2279-2293.

Rosemond, A. D., C. M. Pringle, A. Ramirez, M. J. Paul and J. L. Meyer. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. Limnology and Oceanography 47: 278-289.

Rothfritz, H., I. Jüttner, A. M. Suren and S. J. Ormerod. 1997. Epiphytic and epilithic diatom communities along environmental gradients in the Nepalese Himalaya: implications for the assessment of biodiversity and water quality. Archiv für Hydrobiologie 138: 465-482.

Schindler, D. W., H. Kling, R. V. Schmidt, J. Prokopowich, V. E. Frost, R. A. Reid and M. Capel. 1973. Eutrophication of Lake 227 by addition of phosphate and nitrate: the second, third, and fourth years of enrichment, 1970, 1971, and 1972. Journal of the Fisheries Research Board of Canada 30: 1415-1440.

Slack, N. G. and J. M. Glime. 1985. Niche relationships of mountain stream bryophytes. The Bryologist 88: 7-18.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River Ecosystem to phosphorus fertilization. Ecology 85: 939-954.

Steinman, A. D. 1994. The influence of phosphorus enrichment on lotic bryophytes. Freshwater Biology 31: 53-63.

Steinman, A. D. and H. L. Boston. 1993. The ecological role of aquatic bryophytes in a woodland stream. Journal of the North American Benthological Society 12: 17-26.

Suberkropp, K. and E. Chauvet. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433-1445.

Suren, A. M. 1993. Bryophytes and associated invertebrates in first-order alpine streams of Arthur's Pass, New Zealand. New Zealand Journal of Marine and Freshwater Research 27: 479-494.

Triska, F. J., V. C. Kennedy and R. J. Avanzino 1983. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. *In*

Dynamics of Lotic Ecosystems. T. D. I. Fontaine and S. M. Bartell, eds. Ann Arbor Science Publishers. pp. 129-159.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130-137.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7: 737-750.

Vitousek, P. M., H. A. Mooney, J. Lubchenco and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277: 494-499.

Wallace, J. B., S. L. Eggert, J. L. Meyer and J. R. Webster. 1999. Effects of resource limitaion on a detrital-based ecosystem. Ecological Monographs 69: 409-442.

Wellnitz, T. A., R. B. Rader and J. V. Ward. 1996. Importance of light and nutrients in structuring an algal community in a rocky mountain stream. Journal of Freshwater Ecology 11: 399-413.

Weyers, H. S. and K. Suberkropp. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. Journal of the North American Benthological Society 15: 408-420.

Winterbourn, M. J. 1990. Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. Freshwater Biology 23: 463-474.

CHAPTER 2

NUTRIENTS STIMULATE DETRITAL BREAKDOWN RATES AND DETRITIVORE BIOMASS: BOTTOM-UP EFFECTS VIA HETEROTROPHIC PATHWAYS[1]

[1] Greenwood, J.L., A.D. Rosemond, J.B. Wallace, W.F. Cross and H.S. Weyers. To be submitted to *Ecology*.

Abstract

Most examinations of nutrient enrichment in streams have focused on effects in autotroph-based systems, where algae make up a large proportion of the resource base. We enriched a detritus-based stream, where allochthonous leaf inputs dominate the resource base, with nitrogen and phosphorus to examine the effects on leaf breakdown rate and consumer biomass compared to a control stream. Based on 2 years of pre-treatment and 2 years of enrichment, we determined nutrient effects on breakdown rate, microbial respiration, leaf carbon:nitrogen ratios, invertebrate assemblages and the amount of nitrogen stored in leaves vs. invertebrate biomass on two leaf species differing in quality (carbon:nitrogen), *Rhododendron maximum* and *Acer rubrum* in both a treatment and reference stream. Relative effects were also compared between the two leaf types. Nutrient enrichment significantly accelerated breakdown rates ca. 1.5 and 3 times during the first and second years of enrichment, respectively in the treatment vs. reference stream. Respiration of both leaf types increased about 3-fold with enrichment. Invertebrate biomass associated with leaf packs, particularly shredders, also significantly increased ca. 2-3-times with enrichment. The amount of nitrogen stored in macroinvertebrate biomass relative to leaf standing crop also increased with enrichment up to 44 times for rhododendron leaves and up to 6 times for red maple. Nutrient effects on leaf breakdown and invertebrate response increased during the second year of enrichment relative to the first year. Lower quality rhododendron leaves showed a similar magnitude of response to nutrient enrichment compared to red maple leaves for leaf breakdown, respiration, leaf C:N and invertebrate biomass per leaf pack. Our results indicate that even moderate enrichment of headwater forested streams can result in substantially increased rates of organic matter processing, stimulation of consumer biomass and shifts in elemental balances between resources and consumers. Thus, nutrient enrichment can potentially play a

large role in the stimulation of organic matter processing and energy flow through the detrital food web.

Introduction

Anthropogenic nutrient enrichment can result in profound changes in aquatic food webs and ecosystem function (Carpenter et al. 1998; Smith et al. 1999). Our primary knowledge of nutrient effects in aquatic systems is based on autotrophic pathways through food webs. Algal productivity and biomass in lakes and streams commonly increase due to nutrient enrichment (Elser et al. 1990; Francoeur 2001). Increased primary productivity often results in algal blooms (Carpenter et al. 1998) or can be transformed by herbivory into increased consumer biomass (Peterson et al. 1993). Detritus, however, is also an important energy source in many ecosystem food webs (Hedin 1991). For example, food webs in forested headwater streams are particularly dependent on allochthonous leaves and wood as their primary energy source (Fisher and Likens 1973; Vannote et al. 1980; Wallace et al. 1999). Potentially, nutrient enrichment affects autotrophic and detrital food web components very differently. Nutrient limitation can be widespread in systems with little anthropogenic impact, and nutrient enrichment in algal-based systems typically results in increased autotrophic carbon. However, in detritus-based systems, nutrient enrichment can result in reduced detrital carbon via increased processing rates. For example, leaf breakdown rates are often accelerated by higher concentrations of nitrogen (N) (Meyer and Johnson 1983; Suberkropp and Chauvet 1995), phosphorus (P) (Elwood et al. 1981; Rosemond et al. 2002) or both N and P (Kaushik and Hynes 1971; Howarth and Fisher 1976; Weyers and Suberkropp 1996; Robinson and Gessner 2000; Grattan II and Suberkropp 2001); (Benstead et al. 2004).

We know relatively little about how nutrients affect energy flow to consumers in detritus-based systems compared to autotroph-based systems (Feminella and Hawkins 1995; Brett and Goldman 1997). Detritivores are dependent on both detritus and the microbes associated with detritus for sustenance. Microbial activity on organic material can be enhanced by increasing nutrient concentrations (Howarth and Fisher 1976; Elwood et al. 1981; Suberkropp and Chauvet 1995; Ramirez et al. 2003), resulting in increased quality of detrital food resources for consumers. Increased quality of detrital food resources can potentially result in stimulated growth or production of consumers. Nutrient enrichment of stream water has resulted in increased abundance (Pearson and Connoly 2000; Rosemond et al. 2001) of collectors and biomass of shredding stoneflies (Robinson and Gessner 2000), but relationships between nutrients and detrital consumers are not always clear (Elwood et al. 1981; Meyer and Johnson 1983; Newbold et al. 1983). However, positive bottom-up effects of nutrient enrichment on detrital quality may be counteracted by increased breakdown rates ultimately resulting in a reduction of detrital carbon availability.

In addition to changing the processing dynamics of leaf litter in general, how leaf litter responds to nutrient enrichment should vary with the quality of the leaf species (Webster and Benfield 1986). Differences in leaf quality among leaf species can be represented as differences in leaf breakdown rates. Faster breakdown rates are often related to high amounts of internal nutrients (i.e. lower C:N ratios) and also low amounts of lignin (Melillo et al. 1984; Enriquez et al. 1993; Royer and Minshall 2001; Stelzer et al. 2003). It is feasible that when nutrients are more available in the leaf tissue, microbial activity is less dependent on external nutrient concentrations. Thus, when nutrient supplies are increased in the water column, microbes from leaves with slower breakdown rates should show a stronger response because they depend more

on this external source of N and P (Stelzer et al. 2003). Thus, breakdown rates from these "slow" leaves should increase more relative to "faster" leaves when water column nutrients increase. In fact, (Stelzer et al. 2003) found a higher relative response of low quality substrata (wood) to nutrient enrichment compared to high quality substrata (leaves). However, nutrient enrichment effects on terrestrial leaf breakdown often varies (Hobbie and Vitousek 2000; Aerts et al. 2003), which is considered to be due to low quality carbon components (e.g. lignin) constraining any potential response to added nutrients (Hobbie 2000).

There is clearly support for detrital processing to be limited by stream water nutrients from short-term enrichment studies. However, we know very little about the potential temporal dynamics of detritus processing in forested streams due to chronic enrichment. Long-term studies of nutrient enrichment in detritus-based systems are particularly important considering the potential for increased nutrients to ultimately result in food limitation for detritivores due to increased detrital processing rates. One long-term study of a whole-catchment N enrichment showed little effect on detrital processing due to potential P limitation (Chadwick and Huryn 2003). A long-term P enrichment to a forested stream in Costa Rica showed microbial response as increased respiration, but little effect on insect taxon richness, abundance or biomass (Ramírez 2001). Also, in the highly autotrophic Kuparuk River, AK, long-term P enrichment stimulated mineralization of detritus with low internal phosphorus concentrations, but had no effect on detritus with higher P content (Peterson et al. 1993).

We continuously added N and P to a headwater stream in the southern Appalachian mountains to determine breakdown rates and several other variables compared to a reference stream. In this study, we examined the response of 2 dominant leaf species in the watershed, red maple (*Acer rubrum* L.) and rhododendron (*Rhododendron maximum* L.), which have moderate

and slow breakdown rates, respectively (Paul and Meyer 1996). We predicted that nutrient enrichment would stimulate breakdown rates, microbial activity measured as respiration, decrease C:N content of leaf material, increase invertebrate abundance and biomass, and increase the average amount of N stored in invertebrate tissue relative to leaf material, consistent with a bottom-up response of microbes and consumers to added nutrients. We also predicted that rhododendron would show a stronger response to water column enrichment relative to red maple leaves due to its relatively lower tissue nutrient content (high C:N).

Methods

Study Site

Our study was conducted in two streams located at the Coweeta Hydrologic Laboratory (CHL), a USDA Forest Service research facility located in the Blue Ridge Mountain physiographic province in the southern Appalachian mountains (North Carolina, U.S.A.) from Dec 1998 – July 2002. Both the reference stream (Catchment 53) and the enriched stream (Catchment 54) have similar physical and chemical characteristics (Table 2.1). Dominant vegetation includes tulip poplar (*Liriodendron tulipifera* L.), white oak (*Quercus alba*, L.), red oak (*Quercus rubra*, L.), red maple (*Acer rubrum*, L.), dogwood (*Cornus florida*, L.) and rhododendron (*Rhododendron maximum* L.) (Swank and Crossley 1988).

Nutrient Enrichment

Pretreatment data were collected from both streams from December 1998-July 2000. Beginning 11 July 2000, the treatment stream was continuously enriched with NH_4NO_3 and $KH_2PO_4 + K_2HPO_4$ (in a molar N:P ratio of 11:1 designed to result in a targeted stream water N:P of ca. 15:1) along the entire 150m length of the study reach for two years. A dissolved

nutrient salt solution was pumped into an irrigation line (approx. 2cm diameter) that was fed with stream water from an upstream head tank. The line ran the entire length of study reach adjacent to the streambed while multiple spigots inserted along the length of the hose delivered the nutrient solution into the stream. A metering pump (Liquid Metronics, Inc.) was electronically linked with a Campbell data logger to an ISCO flow measurement device at the downstream end of the stream reach to deliver nutrients in a discharge-dependent manner. Stream water nutrient concentrations were measured during the pretreatment and experimental periods. During the last year of the pretreatment period, one to four samples were taken from the reference and treatment streams on five sampling dates in the reference stream and 12 sampling dates in the treatment stream. During the enrichment period, one sample was taken from the reference stream and five samples were taken along the length of the treatment stream approximately every two weeks to confirm that nutrients were elevated in an even distribution within the study reach. Stream water samples were filtered with Millipore HA filters into acid-washed bottles and frozen until analysis. Concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) were determined with an Alpkem Rapid Flow Analyzer 300 at the University of Georgia Chemical Analysis Laboratory.

Leaf Packs

Single species leaf packs containing either red maple or rhododendron leaves were deployed in the treatment and reference streams to determine breakdown rates. Leaf packs were made with plastic mesh pecan bags (22cm x 40cm, 5mm mesh) to allow access by stream fauna. Freshly-abscised, air-dried leaves of either 5g red maple or 15g rhododendron were used, which resulted in an approximately equal number of leaves of each species per leaf pack. Leaf packs were deployed for a total of 4 seasons on December 2, 1998 and December 14, 1999 (pre-

treatment), and on December 7, 2000 and December 5, 2001 (enrichment period). A new set of leaf packs was deployed each year. Between 3 and 5 red maple packs were retrieved at roughly 7, 14, 28, 55, 100, 135, 160 and 180 days after deployment. Between 3 and 6 rhododendron packs were retrieved at roughly 7, 14, 28, 55, 120, 160, 260, 360 and 390 days after deployment. Leaf packs collected from the field were frozen for later processing.

Material from thawed leaf packs was rinsed, leaf particles >4mm were dried, weighed, and a sub-sample combusted at 500°C to determine remaining ash-free dry mass (AFDM). Leaf breakdown rates were determined using the negative exponential model (Petersen and Cummins 1974). Mass from leaf packs collected with less than 5% of their original weight was not included in the calculation of breakdown rates. Dried leaf material was used for carbon (C) and N elemental analysis. Dried leaves were ball-milled and analyzed for total C and total N by micro-Dumas combustion.

Respiration

Microbial respiration was measured as oxygen uptake during the first 4 sampling dates for rhododendron and 3 sampling dates for red maple of the 2001-2002 season (year 2 of enrichment). Measurements were limited to these dates when leaf material was still intact enough to cut leaf disks. Three replicate leaf packs were used on each date. Ten disks were cut with a #13 cork borer (18mm diameter) from leaf material from each leaf pack. Disks were placed in stream water in a 29mL glass chamber incubated in the stream in the dark. Oxygen concentrations were measured 3x during the 20 minute incubation with a YSI 5100 dissolved oxygen meter using a YSI 5010 self-stirring oxygen probe which capped each chamber (Gulis and Suberkropp 2003). An incubation with only stream water served as a blank. Oxygen consumption was calculated by subtracting the slope of the regression of oxygen concentration

over time of the stream water blank from the regression slope of the sample. The data were expressed per leaf AFDM per hour. The AFDM from the leaf discs from respiration were added back to leaf pack AFDM values for calculating breakdown rates.

Macroinvertebrates

Leaf pack contents were thawed and rinsed over nested sieves (1mm, 250μm) to separate invertebrates from leaf packs and were preserved in 7-8% formalin stained with phloxine-B to facilitate sorting. Insects were identified to the lowest taxonomic level practical, usually genus. Chironomidae were separated into Tanypodinae and non-Tanypodinae. All invertebrates were measured to the nearest 0.5mm to estimate biomass as AFDM using length-weight regressions (Benke et al. 1999). Functional feeding group designations followed (Merritt and Cummins 1996) and our local knowledge of trophic ecology of the benthic invertebrates. Invertebrates were identified for both leaf types on three dates for each of the four years (Year 1: days 14, 70 and 169; Year 2: days 14, 55 and 160; Year 3: days 14, 55 and 170; and Year 4: days 14, 55 and 135). Year 4 used an earlier final date due to the advanced state of breakdown at day 160 in the treatment stream.

Leaf and macroinvertebrate nitrogen storage

How nutrient enrichment affected the relative amount of N contained in invertebrate biomass vs. leaf litter standing crop was assessed. The amount of N contained in the average biomass of invertebrates for the second year of pretreatment and both years of enrichment (from the same dates as macroinvertebrate community analysis) was calculated by assuming that invertebrate biomass was 10% N, as determined from a stoichiometric study from the same study streams (Cross et al. 2003). The amount of N in leaves was determined from the C:N value and dry weight of leaves from individual leaf packs.

Data Analysis

Since only one reference and one experimental stream were used, this experiment was not strictly replicated, violating assumptions of inferential statistics (Hurlbert 1984). However, we feel that the catchment-level spatial scale of the experiment is essential for understanding ecosystem-level effects, and the use of inferential statistics necessary for detection of experimental effects (Oksanen 2001). The slopes of the breakdown rates between reference and enrichment conditions were compared using ANCOVA for each year individually. In order to determine how breakdown rates with enrichment differ from typical breakdown rates under reference conditions, the number of standard deviations between an overall average breakdown rate from long-term data and breakdown rates in the treatment stream during each year of enrichment was calculated. Breakdown rates from the reference stream in this study and from similarly-sized references streams in previous leaf breakdown studies at Coweeta (Wallace et al. 1982; 1986; Cuffney et al 1990; Chung et al. 1993; Whiles and Wallace 1997; (Eggert and Wallace 2003) were used to determine an overall typical average for rhododendron (n=17) and red maple (n=18). Respiration rates were compared with repeated measures MANOVA. Slopes of regression lines for C:N versus number of days in the stream were compared between streams with ANCOVA for each year individually. Only average values of C:N that continued to decrease throughout the leaf pack incubation were included in the analysis. Insect biomass, expressed as per leaf pack and per g AFDM of leaf material remaining in the leaf pack, were examined with repeated measures MANOVA, where pretreatment data were examined separately from enrichment data to determine similarity between streams before and after enrichment. If there was no significant difference between streams before enrichment and there was significant difference between streams after enrichment, this was considered an indication of

a treatment effect. Response of individual invertebrate taxa with >5% overall average relative biomass were also examined with repeated measures MANOVA. The ratio of N contained in macroinvertebrate biomass to N contained in leaf material was compared between streams with a Wilcoxon test. Differences in the relative response of rhododendron and red maple to enrichment was examined by comparing the magnitude of effect in year one and year two of enrichment compared to a reference condition for each leaf type. Breakdown rates in the treatment stream for each year of enrichment were compared with the breakdown rates from both streams average over the 2 years of pre-treatment. For respiration, average values across all dates were compared between streams for the second year of pre-treatment when respiration was measured. For C:N, comparisons were made between slopes of the regression lines between streams and, due to interannual variability, were compared between streams separately for both years of enrichment. As with breakdown rates, measures of invertebrate biomass for each year of enrichment were compared to the overall average of the 2 pre-treatment years.

Results

Nutrient Enrichment

The enrichment increased DIN ~13X to approximately 400µg/L and SRP ~5X to approximately 50 µg/L in the treatment stream (Table 2.2), resulting in a stream water molar N:P ratio of about 18:1. Stock solution was added at a molar N:P ratio of approximately 11:1, which suggests that there was a relatively greater uptake of P. These nutrient concentrations are within range of those found regionally in streams (Scott et al. 2003). Nutrient levels in the reference stream remained consistent across pretreatment and treatment periods with DIN <30µg/L and SRP <10µg/L.

Leaf Breakdown

The leaf breakdown rates for rhododendron were not significantly different between streams in the first year (Fig 2.1 A; ANCOVA df=1, F=0.27, p=0.60) or second year (Fig 2.1B; ANCOVA df=1, F=1.66, p=0.20) of pre-treatment (Table 2.3). However, breakdown rates were significantly faster in the enriched stream compared to the reference stream during the first year (Fig 2.1C; ANCOVA df=1, F=8.03, p<0.01) and the second year (Fig 2.1D; ANCOVA df=1, F=32.43, p<0.0001) of nutrient enrichment (Table 2.3). The overall average breakdown rate and standard deviation for rhododendron from reference streams at Coweeta were 0.0046 and 0.0012 day^{-1}, respectively. Breakdown rates under reference conditions were all within 1.5 standard deviations of the overall average, while year one of enrichment was nearly 3 standard deviations greater and year two of enrichment was over 9 standard deviations greater than the overall average. Further, by the second year of enrichment, days to 95% loss decreased over 3-fold (Table 2.3).

The leaf breakdown rate for red maple was not significantly different between streams in the first year of pre-treatment (Fig. 2.1 E; ANCOVA df=1, F=0.0522, p=0.82), but was slower in the treatment stream the second year (Fig 2.1D; ANCOVA df=1, F=9.34, p=0.003) of pre-treatment (Table 2.3). Similar to effects of treatment on rhododendron, breakdown rates were also significantly faster in the enriched stream compared to the reference stream during the first year (Fig. 2.1G; ANCOVA, df=1, F=17.21, p<0.0001) and the second year (Fig 2.1H; ANCOVA, df=1, F=34.24, p<0.0001) of nutrient enrichment (Table 2.3). The overall average breakdown rate and standard deviation for red maple from reference streams at Coweeta were 0.011 and 0.0034 day^{-1}, respectively. Breakdown rates under reference conditions were all less than 1.5 standard deviations of the overall average, year one of enrichment was nearly 1.5 standard

deviations greater and year two of enrichment was about 4.5 standard deviations greater than the overall average. Additionally, by the second year of enrichment, days to 95% loss decreased nearly 3-fold (Table 2.3).

Respiration

Respiration rates on rhododendron leaves were significantly higher with enrichment (repeated measures MANOVA df=1,4; F=9.77; $p<0.005$), overall averaging 0.06 mg O_2 . g leaf $AFDM^{-1}$. h^{-1} in the treatment stream and 0.2 mg O_2 . g leaf $AFDM^{-1}$. h^{-1} with enrichment (Fig. 2.2A). Respiration rates for maple were also significantly higher in the treatment relative to reference stream (repeated measures MANOVA, df=1,4; F=3.88; $p<0.05$), with an overall average of 0.15 mg O_2 . g leaf $AFDM^{-1}$. h^{-1} in the reference stream and 0.44 mg O_2 . g leaf $AFDM^{-1}$. h^{-1} with enrichment (Fig. 2.2B).

Leaf C:N

The pattern of C:N ratios of leaf material through time changed with enrichment for both rhododendron and red maple (Fig. 2.3). Linear regressions of C:N over time were significant for both rhododendron and red maple during all years at $p<0.0002$. For rhododendron and red maple, the rate of change of C:N was not significantly different between streams during the second year of pre-treatment, the first year C:N was measured (Fig 2.3A & D, Table 2.4). However, slopes of C:N over time were significantly steeper for the treatment stream for both leaf types during the first (Fig. 2.3B & E, Table 2.4) and second (Fig. 2.3C & F, Table 2.4) years of enrichment.

Macroinvertebrates in Rhododendron Leaf Packs

Total biomass of macroinvertebrates measured as both per pack and per g leaf AFDM remaining in rhododendron leaf packs was similar between streams before enrichment and increased in the treatment stream during enrichment (Figs. 2.4A & E, Table 2.5). When analyzed

separately by date, total invertebrate biomass was similar between streams before enrichment, but was only significantly different between streams on day 160 measured as both per pack and per g leaf AFDM remaining (Figs 2.4B-D & F-H, Table 2.5) after enrichment. A similar pattern to the annual average total biomass was also seen for annual average biomass of shredders (the biomass dominant), gatherers, and total primary consumers (i.e. non-predators) (Figs. 2.4A & E, Table 2.5). Annual average biomass of filterers and predators showed no significant differences between streams either before or during enrichment (Figs 2.4A & E, Table 2.5). The 5 taxa tested for nutrient effects, *Fattigia pele*, *Pycnopsyche* spp., *Tallaperla* spp., *Tipula* spp., and *Lanthus* spp., made up, on average, ca. 60% of the total biomass (Table 2.6). Only the shredding trichopteran, *Pycnopsyche* spp. increased in total biomass in the treatment stream after enrichment, and there was no difference between streams before enrichment (Table 2.6). *Tipula* spp. was the only other taxon to show significant differences between streams, and for this taxon biomass levels were higher in the reference stream before enrichment, but higher in the treatment stream after enrichment (Table 2.6).

Macroinvertebrates in red maple leaf packs

Total biomass of macroinvertebrates measured as both per pack and per g leaf AFDM remaining in red maple leaf packs was similar between streams before enrichment and increased in the treatment stream during enrichment (Figs. 2.5A & E, Table 2.7). For individual dates, total invertebrate biomass was similar between streams before enrichment, and was significantly different between streams on a per pack basis on Day 14 (Fig. 2.5B), and on a per g leaf AFDM remaining basis, differences were significant on both Day 14 (Fig 2.5G) and Day 55 (Fig. 2.5H). A similar pattern to the annual average total biomass was also seen for biomass of shredders (the biomass dominant), gatherers, and total primary consumers (Fig. 2.5A & E, Table 2.7). Biomass

of filterers was significantly different between streams before nutrient enrichment, but not after (Fig. 2.5A & E, Table 2.7), and predator biomass showed no significant differences between streams either before or during enrichment (Fig. 2.5A & E, Table 2.7). The 5 taxa tested for nutrient effects, *Lepidostoma* spp., *Pycnopsyche* spp., *Tallaperla* spp., *Tipula* spp., and *Lanthus* spp., made up, on average, ca. 60% of the total biomass (Table 2.8). Only the shredding trichopteran *Lepidostoma* spp. increased in total biomass in the treatment stream after enrichment, and there was no difference between streams before enrichment. The predatory odonate, *Lanthus* spp., was the only other taxon to show significant differences between streams, and for this taxon, biomass levels were higher in the reference stream before enrichment, with no differences between streams after enrichment (Table 2.8).

Invertebrate assemblages were similar between leaf types regarding distribution of functional feeding groups (Figs. 2.4 and 2.5). The dominant species for both leaf types were also similar except for a trade-off between dominance of *Fattigia pele* in rhododendron and *Lepidostoma* spp. in red maple (Table 2.6 and 2.8). Red maple tended to support more invertebrate biomass per leaf mass compared to rhododendron, generally between 3X and 35X more biomass per leaf mass compared to rhododendron (Figs. 2.4 and 2.5).

Leaf and macroinvertebrate nitrogen storage

While C:N of leaf packs in the treatment stream was declining relative to the reference, invertebrate biomass and presumably the storage pool of invertebrate-associated N was increasing. Thus, we felt it useful to quantify the potential shift in N storage in leaves vs. consumers for a given date. The ratio of invertebrate total N to leaf total N per leaf pack was similar between streams during the second year of pretreatment (first year of pretreatment was not tested) for rhododendron and red maple (Fig. 2.6A & B; Wilcoxon z=0.66, p=0.43). During

the first year of enrichment, ratios were marginally significantly different between streams for rhododendron (Wilcoxon z=1.85, p=0.06), but not significantly different for red maple (Wilcoxon z=71; P=0.48). There was a not a significant difference during the second year of enrichment for rhododendron (Wilcoxon z=1.59, p=0.11), however, there was a trend of a much higher ratio of invertebrate N to leaf N during the second year of enrichment relative to the other years. Red maple did exhibit a significant difference between streams during the second year of enrichment (Wilcoxon z=2.54, p=0.01).

Relative response of rhododendron and red maple to nutrient enrichment

Rhododendron and red maple generally displayed similar magnitudes of response to enrichment, but at times the stronger response would alternate between the 2 leaf types across the 2 years of enrichment (Table 2.9). However, if either rhododendron or red maple showed a stronger response, the difference between leaf types tended to be much greater when rhododendron exhibited the stronger response. Also, when there was a difference in magnitude change for a variable between the 2 years of enrichment, it was almost always higher during the second year of enrichment. Breakdown rates changed similarly during the first year of enrichment between rhododendron and red maple, where breakdown rates increased 1.6 and 1.7-fold with enrichment, respectively. During the second year of enrichment, breakdown rates for rhododendron increased slightly more than 3-fold and increased for maple slightly less than 3-fold in the enriched stream. Respiration rates roughly tripled for both leaf types with enrichment (Table 2.9). Red maple showed a similar magnitude decrease in C:N during both years of enrichment, about 2.5 fold. Rhododendron C:N, on the other hand, only decreased 2-fold in the first year of enrichment, but decreased nearly 4-fold during the second year (Table 2.9). The increase in invertebrate biomass per leaf pack was fairly similar for both leaf types, increasing

about 2-fold the first year and 3-fold the second year of enrichment, although the change was slightly higher for rhododendron the first year of enrichment and slightly higher for red maple during the second year of enrichment. However, the magnitude change in invertebrate biomass per g leaf AFDM remaining was greater for red maple during the first year of enrichment (11-fold vs. nearly 7-fold), but greater for rhododendron during the second year of enrichment (14-fold vs. 72-fold). Rhododendron showed a greater magnitude change for both years of enrichment relative to red maple in the ratio of total invertebrate N to total leaf N, increasing 6-fold and 44-fold compared to the increase in red maple of about 2-fold and 6-fold.

Discussion

Evidence for nutrient limitation on detrital processing and leaf pack-associated biota

An increasing number of studies have begun to test nutrient enrichment effects on detrital-based food web components (Ramirez 2001; Rosemond et al. 2001; Rosemond et al. 2002; Chadwich and Huryn 2003; Steltzer et al 2003). Our study showed clear effects of nutrient stimulation on microbes associated with leaf litter, the dominant food resource to consumers in our study streams. Increased microbial activity and biomass presumably influenced quality of detritus (measured as C:N) and resulted in greater biomass of invertebrates per unit detrital carbon with nutrient enrichment. These bottom-up effects were profound, with enrichment resulting in up to 3X the biomass of invertebrates supported on leaf litter in the treatment stream relative to the reference. Processing rates of leaf litter were thus stimulated under nutrient enrichment, presumably via both microbial activity and invertebrate consumption. These results illustrate ways that nutrient enrichment effects are manifested resulting in reduced quantity of detrital carbon, but increased quality, which had positive effects on standing stock of consumers.

The bulk of other studies examining nutrient enrichment effects on heterotrophs and consumers have similarly found increased microbial biomass or activity (Elwood et al. 1981; Suberkropp and Chauvet 1995; Ramirez et al. 2003) and positive effects on metazoan detritivores (Pearson and Connoly 2000; Robinson and Gessner 2000; Rosemond et al 2001). Our study provides additional evidence that nutrient enrichment of aquatic systems can have profound effects via heterotrophic pathways.

Microbial vs. invertebrate control of increased processing due to nutrient addition

Measurements of leaf breakdown integrate several factors which contribute to detrital processing rates. The dominant biological factors, microbial and invertebrate activity, are particularly important (Boulton and Boon 1991). We have evidence that positive effects of enrichment on both microbial activity and invertebrate biomass most likely led to the higher measures of leaf breakdown rates. Our measurements of respiration indicated that microbial activity was enhanced on leaves. Also, microbial activity and breakdown rates have been shown to increase with nutrient enrichment on wood substrata from the same stream (Gulis et al. 2004) and another Coweeta stream (Tank and Webster 1998). In a study of breakdown of red maple and rhododendron leaves in the same study streams during year 1 of nutrient enrichment, breakdown rates increased with enrichment in smaller mesh, invertebrate-excluding leaf packs (Gulis and Suberkropp 2003), indicating that microbial processing is at least partially responsible for the increase in breakdown rates. However, these breakdown rates were not quite as high as rates reported from this study (about 50% of our rhododendron breakdown rates, and about 80% of red maple) with leaf packs that did not exclude most invertebrates, indicating that invertebrate activity is also an important additional contributor to leaf breakdown rates. Several studies have also shown higher breakdown rates in course-mesh leaf packs vs. small-mesh leaf packs in low

order streams (Robinson and Gessner 2000; Graca et al. 2001; Rosemond et al. 2002). It should be noted that larger mesh leaf packs may be prone to more physical abrasion (Webster and Benfield 1986) which should not be discounted as a factor affecting breakdown rates. However, due to the similar physical features of our study streams, it is not likely that physical abrasion contributed to the differences seen between streams in this study. Physical abrasion may have played a role in interannual differences in breakdown rates in for rhododendron. Breakdown rates of rhododendron were unusually high during the second year of pre-treatment in both the treatment and reference streams. Discharge may have played a role in this phenomenon. Drought conditions prevailed throughout most of the study period, but the highest average discharge for the entire study period occurred in April 2000 which was 5X the overall average. However, a similar effect on leaf breakdown was not seen for red maple leaf packs during that same year.

Increased food quality related to microbial biomass was likely an important factor influencing the positive response of invertebrates to nutrient enrichment in this study. Although leaf-associated microbes can directly take up stream water nutrients (Weyers and Suberkropp 1996; Suberkropp 1998; Sridhar and Bärlocher 2000; Grattan II and Suberkropp 2001), resulting in enhanced microbial biomass (Kaushik and Hynes 1971; Lawson et al. 1984; Bärlocher 1985), macroinvertebrates must obtain these nutrients indirectly. Therefore, the increase in invertebrate biomass that we saw in leaf packs was probably largely due to invertebrates consuming a more nutritious food resource. We measured this increase in quality as a decrease in leaf C:N ratio which, presumably was a result of amplified microbial biomass on leaf material. In a concurrent study, growth rates and survivorship for several taxa also increased in the enriched stream (Cross 2004), supporting the view that invertebrates were consuming more nutritious food. In spite of the increase in food quality, it seems likely that the increased rates of detrital processing will

ultimately result in food limitation since the length of time during the year that leaf litter is available in the study reach has been truncated. Under ambient nutrients rhododendron leaves would not reach 95% loss for nearly 2 years, but with enrichment, the same result is reached in less than 1 year. Similarly, where red maple leaves would reach 95% loss in nearly a year, the same result is reached in less than 6 months. Thus, the nutrient effect on leaf availability may be particularly important for invertebrates, particularly longer-lived detrital consumers, in summer when most other leaf types are relatively unavailable and detritivores would normally depend more on recalcitrant leaf types such as rhododendron (Schofield et al. 2001).

Several times more N is being stored in invertebrate biomass relative to leaf tissue with nutrient enrichment. This means that the rate of N cycling in the streams has increased, and the N is being transferred to consumers from resources more efficiently. Thus the rate of flow of N through the food web has accelerated. Also, evidence suggests that in the stream overall, more nitrogen is being stored in consumers relative to leaf biomass. Annual average leaf litter standing crop in the stream has decreased about one-third (K. Suberkropp, unpubl. data), and stream invertebrate biomass has roughly doubled with enrichment (Cross 2004).

In this study, the invertebrate assemblage composition and response to nutrient enrichment was very similar between leaf types, although red maple was able to support more biomass relative to rhododendron. Overall, the bottom-up effect of nutrient enrichment on the invertebrate community in leaf packs was seen primarily as an increase in shredder biomass in leaf packs for both leaf types. The increase in shredders is not surprising since they are often found to be important contributors to leaf breakdown in streams from Coweeta (Cuffney et al. 1990; Chung et al. 1993; Eggert and Wallace 2003) and elsewhere (Pearson and Connoly 2000; Robinson and Gessner 2000; Haapala et al. 2001; Hieber and Gessner 2002). Among gatherers, filterers and

predators, only gatherers showed a significant response to nutrient enrichment. Gatherers could benefit from enhanced availability of fine particulate organic matter due to the increased leaf processing or fungi that permeate leaves, which we know to be the primary response of the microbial community to nutrient enrichment in this study (Suberkropp unpubl. data). Filterers and predators made up relatively small proportions of the biomass in leaf packs, most likely as a function of habitat preference, which would make detecting nutrient effects difficult. In contrast, in samples from mixed substrate habitat, all functional feeding groups showed significant increases in abundance and biomass with nutrient enrichment while at the same time maintaining similar contribution to relative biomass (Cross 2004). Thus, while filterer and predator biomass was not affected by nutrient enrichment in leaf packs, it nonetheless showed a positive response on a larger spatial scale.

Role of leaf quality in response to nutrient enrichment

Leaf quality appeared to play a role in determining the potential response of some variables to nutrient enrichment. Generally, red maple showed characteristics of a higher quality leaf type with breakdown rates about two-thirds faster, respiration rates 2-4 times faster and invertebrate biomass per leaf pack 2-50 times higher compared to rhododendron under reference conditions. With enrichment, rhododendron and red maple leaves showed surprisingly similar responses to several variables: breakdown rate, respiration, leaf C:N and invertebrate biomass per leaf pack. However, lower quality wood substrata showed a stronger response to nutrient enrichment compared to higher quality leaves during this stream enrichment for C:N and breakdown rate (Gulis et al. 2004). Additionally, the difference in response between the two leaf types in this study was much lower than differences seen between leaves and wood in the above studies (wood or wood veneers responded at least twice as much as leaves to enrichment). Potentially,

the difference in quality of heterotrophic substrata needs to be much greater to detect differential effects of external nutrient enrichment. Also, wood may generally be less competitive for low levels of external nutrients relative to leaves (Tank and Webster 1998), which may explain the relatively greater response of wood to nutrient enrichment relative to leaves. However, rhododendron more consistently showed a stronger response compared to red maple in invertebrate biomass per leaf AFDM and the ratio of invertebrate total N to leaf total N. This may reflect a potentially greater importance of invertebrates in the processing of rhododendron leaves relative to red maple. The relative importance of invertebrate processing for rhododendron was also seen in an earlier study of invertebrate removal via insecticides in these streams, where a greater treatment effect was also seen on breakdown rates of rhododendron compared to red maple (Wallace et al. 1982; Chung et al. 1993).

Long-term effects

P seems to be more important than N in driving the overall response seen in this study to nutrient enrichment. Our data suggest that P was preferentially taken up over N, since our nutrient enrichment solution was added at a molar N:P ratio of approximately 11:1, and the resulting ratio in the enriched stream was 18:1. Other studies comparing nitrogen and phosphorus uptake in streams at Coweeta have also shown greater uptake of P relative to N (Munn and Meyer 1990; Webster et al. 1991)

The second year of nutrient enrichment exhibited greater effects on response variables, particularly leaf breakdown and invertebrate biomass, compared to the first year. This indicates that the system has not yet reached a new equilibrium with the higher levels of N and P. The difference between Year 1 and Year 2 of enrichment could be mostly a result of an additional increase in invertebrate activity during Year 2. Microbial biomass and production did not

considerably differ between the two years of enrichment (K. Suberkropp, unpublished data). However, invertebrate biomass from this study, and invertebrate secondary production from mixed substrate (Cross 2004) was roughly 20% higher during the second year of enrichment. This additional increase in the second year may result from a time lag in bottom-up effects on invertebrates. There is evidence that survivorship of invertebrates increased and there could also be enhanced fecundity due to a larger final size of female instars (Cross 2004), factors which would both manifest during the second year of enrichment. There is further evidence to suggest that even after 2 years of enrichment, an equilibrium state had not been reached with the experimental nutrient concentrations. In a continuation of the nutrient enrichment experiment, leaf breakdown rates for both rhododendron and red maple increased in the third year and again in the fourth year of enrichment (A.D. Rosemond, unpubl. data). It is likely that the ultimate effects of enrichment may not be known for several more years. This may not be an unusual phenomenon. In another long-term stream enrichment studies, the major modification of an Alaskan stream ecosystem from P enrichment was ultimately due to a dramatic increase of benthic bryophyte biomass (Slavik et al. 2004) and took 7 years of enrichment to occur (Bowden et al. 1994). Although our two years of enrichment is a relatively long study, and we saw strong effects of nutrient enrichment on leaf litter processing, an increasing magnitude of nutrient effects are still being seen in this stream, and the ultimate effects of enrichment are still to be determined.

Our results may help explain results seen in some studies of stream disturbance. Several studies have shown increased leaf and wood breakdown rates in streams from a clearcut catchment at Coweeta (Webster and Waide 1982; Golladay and Webster 1988; Benfield et al. 2001). Increased nitrate levels were measured in this stream after clearcutting, which could have

played a role in the acceleration of breakdown rates. However, the exact role nutrients play in influencing breakdown rates after a clear cut is not well understood, since several other factors may also be involved in accelerating breakdown rates due to clearcutting, such as increased temperature, discharge, sediment transport and invertebrate feeding.

Even with relatively moderate levels of nutrient enrichment, we have shown fundamental changes in detrital processing in headwater forested streams. In contrast with autotroph-based systems, nutrient enrichment has accelerated carbon loss as organic matter breakdown. Also, while the timing of availability of leaf litter as a food resource has been compressed, the quality of leaf litter as a food resource has increased. Just as nutrient enrichment in autotroph-based systems can result in greater herbivore biomass, we have also shown positive bottom-up nutrient effects on detrital consumers. The effects of nutrient enrichment, however, have not reached equilibrium after 2 years of continuous enrichment. Thus, ecosystem function in southern Appalachian forested headwater streams is potentially very sensitive to nutrient loading, and impacts of nutrient enrichment may show increasing effects over time.

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References

Aerts, R., H. de Caluwe and B. Beltman. 2003. Plant community mediated vs. nutritional controls on litter decomposition rates in grasslands. Ecology 84: 3198-3208.

Bärlocher, F. 1985. The role of fungi in the nutrition of stream invertebrates. Botanical Journal of the Linnean Society 91: 83-94.

Benke, A. C., A. D. Huryn, L. A. Smock and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North American with particular reference to the southeastern United States. Journal of the North American Benthological Society 18: 308-343.

Benstead, J. P., L. A. Deegan, B. J. Peterson, A. D. Huryn, W. B. Bowden, K. Suberkropp, K. M. Buzby, A. C. Green and J. A. Vacca. 2004. Responses of a beaded arctic stream to short-term N and P fertilisation. Freshwater Biology In Review.

Boulton, A. J. and P. I. Boon. 1991. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? Australian Journal of Marine and Freshwater Research 42: 1-43.

Bowden, W. B., J. C. Finlay and P. E. Maloney. 1994. Long-term effects of PO4 fertilization on the distribution of bryophytes in an arctic river. Freshwater Biology 32: 445-454.

Brett, M. T. and C. R. Goldman. 1997. Consumer versus resource control in freshwater pelagic food webs. Science 275: 384-386.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley and V. H. Smith. 1998. Nonpoint polllution of surface waters with phosphorus and nitrogen. Ecological Applications 8: 559-568.

Chadwick, M. A. and A. D. Huryn. 2003. Effect of a whole-catchment N addition on stream detritus processing. Journal of the North American Benthological Society 22: 194-206.

Chung, K., J. B. Wallace and J. W. Grubaugh. 1993. The impact of insecticide treatment on abundance, biomass and production of litterbag fauna in a headwater stream: a study of pretreatment, treatment and recovery. Limnologica 28: 93-106.

Cross, W. F. 2004. Nutrient enrichment of a detritus-based stream ecosystem: effects on invertebrate community structure and function. Dissertation. University of Georgia Athens, GA, USA.

Cross, W. F., J. P. Benstead, A. D. Rosemond and J. B. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6: 721-732.

Cuffney, T. F., J. B. Wallace and G. J. Lugthart. 1990. Experimental evidence quantifiying the role of benthic invertebrates in organic matter dynamics of headwater streams. Freshwater Biology 23: 281-299.

Eggert, S. L. and J. B. Wallace. 2003. Litter breakdown and invertebrate detritivores in a resource-depleted Appalachian stream. Archiv für Hydrobiologie 156: 315-338.

Elser, J. J., E. R. Marzolf and C. R. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences 47: 1468-1477.

Elwood, J. W., J. D. Newbold and A. F. Trimble. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146-158.

Enriquez, S., C. M. Duarte and K. Sand-Jensen. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457-471.

Feminella, J. W. and C. P. Hawkins. 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. Journal of the North American Benthological Society 14: 465-509.

Fisher, S. G. and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs 43: 421-439.

Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society 20: 358-368.

Graca, M. A. S., R. C. F. Ferreira and C. N. Coimbra. 2001. Litter processing along a stream gradient: the role of invertebrates and decomposers. Journal of the North American Benthological Society 20: 408-420.

Grattan II, R. M. and K. Suberkropp. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. Journal of the North American Benthological Society 20: 33-43.

Gulis, V. and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48: 123-134.

Haapala, A., T. Muotka and A. Markkola. 2001. Breakdown and macroinvertebrate and fungal colonization of alder, birch, and willow leaves in a boreal forest stream. Journal of the North American Benthological Society 20: 395-407.

Hedin, L. O. 1991. Cross-System Comparisons of Detritus Food Webs. *In* Comparative Analyses of Ecosystems: Patterns, Mechanisms, and Theories. J. Cole, G. Lovett and S. Findlay, eds. New York, Springer-Verlag. pp. 346-347.

Hieber, M. and M. O. Gessner. 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83: 1026-1038.

Hobbie, S. E. 2000. Interactions between Litter Lignin and Soil Nitrogen Availability during Leaf Litter Decomposition in a Hawaiian Montane Forest. Ecosystems 3: 484-494.

Hobbie, S. E. and P. M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81: 1867-1877.

Howarth, R. W. and S. G. Fisher. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. Freshwater Biology 6: 221-228.

Kaushik, N. K. and H. B. N. Hynes. 1971. The fate of dead leaves that fall into streams. Archiv für Hydrobiologie 68: 465-515.

Lawson, D. L., M. J. Klug and R. W. Merritt. 1984. The influence of the physical, chemical, and microbiological characteristicsof decomposing leaves on the growth of the detritivore *Tipula abdominalis* (Diptera: Tipulidae). Canadian Journal of Zoology 62: 2339-2343.

Melillo, J. M., R. J. Naiman, J. D. Aber and A. E. Linkins. 1984. Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bulletin of Marine Science 35: 341-356.

Merritt, R. W. and K. W. Cummins, Eds. 1996. An Introduction to the Aquatic Insects of North America. Dubuque, IA, Kendall/Hunt Publishing Company.

Meyer, J. L. and C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. Freshwater Biology 13: 177-183.

Newbold, J. D., J. W. Elwood, M. S. Schulze, R. W. Stark and J. C. Barmeier. 1983. Continuous ammonium enrichment of a woodland stream: uptake kinetics, leaf decomposition, and nitrification. Freshwater Biology 13: 193-204.

Paul, M. J. and J. L. Meyer. 1996. Fungal biomass of 3 leaf litter species during decay in an Appalachian stream. Journal of the North American Benthological Society 15: 421-432.

Pearson, R. G. and N. M. Connoly. 2000. Nutrient enhancement, food quality and community dynamics in a tropical rainforest stream. Freshwater Biology 43: 31-42.

Petersen, R. C. and K. W. Cummins. 1974. Leaf processing in a woodland stream. Freshwater Biology 4: 343-368.

Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. McKinely, M. C. Miller, J. R. Vestal, R. Ventullo and G. Volk. 1993. Biological responses of a tundra river to fertilization. Ecology 74: 653-672.

Ramírez, A. 2001. Control of benthic assemblages in detritus-based tropical streams. Ph.D. Dissertation. University of Georgia Athens, Georgia, USA.

Ramirez, A., C. M. Pringle and L. Molina. 2003. Effects of stream phosphorus levels on microbial respiration. Freshwater Biology 48: 88-97.

Robinson, C. T. and M. O. Gessner. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia 122: 258-263.

Rosemond, A. D., C. M. Pringle, A. Ramirez and M. J. Paul. 2001. A test of top-down and bottom-up control in a detritus-based food web. Ecology 82: 2279-2293.

Rosemond, A. D., C. M. Pringle, A. Ramirez, M. J. Paul and J. L. Meyer. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. Limnology and Oceanography 47: 278-289.

Royer, T. V. and G. W. Minshall. 2001. Effects of nutrient enrichment and leaf quality on the breakdown of leaves in a hardwater stream. Freshwater Biology 46: 603-610.

Schofield, K. A., C. M. Pringle, J. L. Meyer and A. B. Sutherland. 2001. The importance of crayfish in the breakdown of rhododendron leaf litter. Freshwater Biology 46: 1191-1204.

Scott, M. C., G. S. Helfman, M. E. McTammany, E. F. Benfield and P. Bolstad, V. 2003. Multiscale influences on physical and chemical stream conditions across Blue Ridge landscapes. Journal of the American Water Resources Association 38: 1379-1392.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River Ecosystem to phosphorus fertilization. Ecology 85: 939-954.

Smith, V. H., G. D. Tilman and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100: 179-196.

Sridhar, K. R. and F. Bärlocher. 2000. Initial Colonization, Nutrient Supply, and Fungal Activity on Leaves Decaying in Streams. Applied and Environmental Microbiology 66: 1114-1119.

Stelzer, R. S., J. Heffernan and G. E. Likens. 2003. The influence of dissolved nutrients and particulate organic matter quality on microbial respiraiton and biomass in a forest stream. Freshwater Biology 48: 1925-1937.

Suberkropp, K. 1998. Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. Mycological Research 8: 998-1002.

Suberkropp, K. and E. Chauvet. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433-1445.

Swank, W. T. and D. A. Crossley 1988. Forest Hydrology and Ecology at Coweeta. Springer-Verlag. New York. 469 pp.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130-137.

Wallace, J. B., S. L. Eggert, J. L. Meyer and J. R. Webster. 1999. Effects of resource limitaion on a detrital-based ecosystem. Ecological Monographs 69: 409-442.

Webster, J. R. and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17: 567-594.

Weyers, H. S. and K. Suberkropp. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. Journal of the North American Benthological Society 15: 408-420.

Table 2.1. Physical and chemical characteristics of the reference stream and enriched stream from CHL. Discharge, and temperature are from July 1999-July 2002. Nutrient and pH data are from the pretreatment period July 1999- July 2000.

		Reference	Enriched
Catchment	Area (ha)	5.2	5.5
	Elevation (m a.s.l.)	820	841
Channel	Gradient (cm m^{-1})	27	33
	Length (m)	145	282
	Bankfull area (m^2)	327	443
Temperature (°C)	Daily mean (*n*)	12.0 (336)	12.0 (336)
	Range	2.6-18.6	4.8-16.7
Discharge (L s^{-1})	Daily mean (*n*)	0.32 (1114)	0.53 (1114)
	Range	0.006-3.8	0.06-4.8
pH	Mean (*n*)	6.59 (24)	6.87 (18)
	Range	6.2-7.0	6.6-7.9
NO_3-N (μg L^{-1})	Mean (*n*)	15.4 (5)	18.8 (12)
	Range	9.4-25.8	4.0-39.5
NH_4-N (μg L^{-1})	Mean (*n*)	9.4 (4)	9.9 (12)
	Range	0-30.4	0-24.9
SRP (μg L^{-1})	Mean (*n*)	7.6 (5)	8.8 (12)
	Range	0-20.3	0-22.1

Table 2.2. Average pretreatment (July 1999 – July 2000) and treatment (July 2000 – July 2002) nitrite + nitrate nitrogen ($(NO_2^-+NO_3^-)$-N), ammonium nitrogen ($NH4^+$-N) and soluble reactive phosphorus (SRP) concentrations ($\mu g\ L^{-1}$) in the reference and treatment streams. Numbers in parentheses are one standard deviation. BD = below detection.

Site	$(NO_2^-+NO_3^-)$-N	$NH4^+$-N	SRP	Molar DIN:SRP
Pretreatment				
Reference				
Mean n=5	15.4(6.6)	9.4(14.1)	7.6(8.0)	7.2
Range	9-26	BD-30	BD-20	
Treatment				
Mean n=12	18.8(11.5)	9.9(8.6)	8.8(8.1)	7.2
Range	4-40	BD-25	BD-22	
Treatment				
Reference				
Mean n=33	16.9(29.8)	10.4(16.9)	3.7(4.7)	16.3
Range	BD-151	BD-76	BD-17	
Treatment				
Mean n=44	308.9(377.8)	105.5(119.7)	51.2(55.6)	17.9
Range	11-1711	6-566	BD-268	

Table 2.3. Values during the 2 years of pre-treatment and 2 years of treatment for breakdown rates (-*k*) for rhododendron (*Rhododendron maximum*) and red maple (*Acer rubrum*) with 1 SE of the slope, the difference between the treatment and reference stream for -*k*, and r^2 of the regression line. All p-values for the regression lines were <0.0001.

	-*k*	SE	Tmt *k* – Ref *k*	r^2	Days to 95% Loss
Rhododendron					
Reference Stream					
Pre-Tmt Yr 1	0.0033	0.00033	+0.0002	0.77	916
Pre-Tmt Yr 2	0.0064	0.00063	-0.0001	0.79	465
Treatment Yr 1	0.0037	0.00059	+0.004	0.58	820
Treatment Yr 2	0.0043	0.00045	+0.0114	0.72	693
Treatment Stream					
Pre-Tmt Yr 1	0.0035	0.00028		0.85	858
Pre-Tmt Yr 2	0.0063	0.00071		0.76	477
Treatment Yr 1	0.0077	0.00068		0.87	388
Treatment Yr 2	0.0157	0.00010		0.93	190
Red Maple					
Reference Stream					
Pre-Tmt Yr 1	0.0100	0.00119	+0.0009	0.68	299
Pre-Tmt Yr 2	0.0098	0.00093	-0.0032	0.77	305
Treatment Yr 1	0.0093	0.00011	+0.0070	0.76	324
Treatment Yr 2	0.0085	0.00087	+0.0177	0.75	351
Treatment Stream					
Pre-Tmt Yr 1	0.0109	0.00013		0.63	276
Pre-Tmt Yr 2	0.0066	0.00051		0.83	453
Treatment Yr 1	0.0163	0.00100		0.93	184
Treatment Yr 2	0.0262	0.00222		0.91	114

Table 2.4. Slopes of C:N through time for rhododendron (*Rhododendron maximum*) and red maple (*Acer rubrum*) leaves in the reference (Ref) and treatment (Tmt) streams, and results of ANCOVA comparison (df=1) of slopes between the treatment and reference streams for regression lines of C:N through time for rhododendron and red maple. The reference and treatment streams were compared for each year individually. Values in boldface are <0.05.

	Ref Slope	Tmt Slope	F	p
Rhododendron				
Pre-Treatment	-0.17	-0.24	3.6733	0.06
N+P Year 1	-0.33	-0.63	13.613	**0.0006**
N+P Year 2	-0.29	-0.88	10.1264	**0.003**
Red Maple				
Pre-Treatment	-0.30	-0.32	0.0678	0.80
N+P Year 1	-0.37	-0.95	28.029	**<0.0001**
N+P Year 2	-0.23	-0.52	10.935	**0.002**

Table 2.5. Results of repeated measured MANOVA (df=1,4) of invertebrate biomass for rhododendron (*Rhododendron maximum*) leaf packs between streams measured as both per leaf pack and per g leaf AFDM remaining in leaf packs. Values in boldface are <0.05. Data were log (x+1) transformed. (+) indicates direction of effect in treatment vs. reference stream.

	Before Enrichment		After Enrichment	
Invertebrate Group	F	p	F	p
Total Invertebrate Biomass per Leaf Pack				
Annual Average	0.14	0.49	7.19	(+) **0.006**
Day 14	2.76	0.17	4.45	0.10
Day 55	1.18	0.34	2.68	0.18
Day 160	1.01	0.37	10.67	(+) **0.03**
Total Invertebrate Biomass per g leaf AFDM remaining				
Annual Average	0.01	0.55	27.32	(+) **0.02**
Day 14	2.84	0.17	4.21	0.11
Day 55	1.57	0.28	3.71	0.13
Day 130	1.46	0.29	31.90	(+) **0.005**
Annual Invertebrate Biomass per Leaf Pack				
Shredders	0.044	0.70	18.93	(+) **0.001**
Gatherers	0.17	0.46	2.49	(+) **0.03**
Filterers	0.14	0.50	0.04	0.71
Primary consumers	0.04	0.72	8.94	(+) **0.004**
Predators	0.10	0.57	0.04	0.70
Annual Invertebrate Biomass per g leaf AFDM remaining				
Shredders	0.07	0.62	50.29	(+) **0.0001**
Gatherers	0.16	0.47	3.49	(+) **0.02**
Filterers	0.64	0.18	1.18	0.10
Primary consumers	0.15	0.49	35.88	(+) **0.0003**
Predators	0.11	0.57	3.58	0.70

Table 2.6. Average percent biomass and results of repeated measured MANOVA (df=1,4) of invertebrate biomass taxa >5% overall average biomass per rhododendron (*Rhododendron maximum*) leaf pack between streams. P-values in boldface are <0.05. Data were log (x+1) transformed. (+) indicates direction of effect in treatment vs. reference stream.

	Before Enrichment		After Enrichment	
Invertebrate Taxon	F	p	F	p
Shredder				
Trichoptera				
Fattigia pele	0.009	0.86	0.03	0.76
Pycnopsyche spp.	0.20	0.43	3.85	(+) **0.02**
Plecoptera				
Tallaperla spp.	0.39	0.28	0.62	0.19
Diptera				
Tipula spp.	2.59	(-) **0.03**	2.04	(+) **0.04**
Predator				
Odonata				
Lanthus spp.	0.64	0.19	1.06	0.11

Table 2.7. Results of repeated measured MANOVA (df=1,4) of invertebrate biomass for red maple (*Acer rubrum*) leaf packs between streams measured as both per leaf pack and per g leaf AFDM remaining in leaf packs. Values in boldface are <0.05. Data were log (x+1) transformed. (+) indicates direction of effect in treatment vs. reference stream.

	Before Enrichment		After Enrichment	
Invertebrate Group	F	p	F	p
Total Invertebrate Biomass per Leaf Pack				
Annual Average	0.16	0.47	6.22	(+) **0.02**
Day 14	6.44	0.06	23.18	(+) **0.02**
Day 55	1.69	0.26	3.74	0.13
Day 130	4.96	0.09	0.0005	0.98
Total Invertebrates per g leaf AFDM remaining				
Annual Average	0.03	0.73	3.00	(+) **0.03**
Day 14	6.06	0.07	23.98	(+) **0.008**
Day 55	1.30	0.32	31.03	(+) **0.005**
Day 130	5.53	0.08	0.777	0.42
Annual Invertebrate Biomass per Leaf Pack				
Shredders	0.005	0.88	7.79	(+) **0.02**
Gatherers	1.05	0.11	9.21	(+) **0.01**
Filterers	2.58	(+) **0.03**	1.28	0.14
Primary consumers	0.33	0.32	9.98	(+) **0.01**
Predators	0.0009	0.95	0.007	0.89
Annual Invertebrate biomass per g leaf AFDM remaining				
Shredders	0.04	0.72	3.13	(+) **0.02**
Gatherers	0.68	0.17	2.08	(+) **0.04**
Filterers	2.30	(+) **0.04**	0.29	0.34
Primary consumers	0.13	0.50	3.23	(+) **0.02**
Predators	0.34	0.31	0.25	0.38

Table 2.8. Average percent biomass and results of repeated measured MANOVA (df=1,4) of invertebrate biomass taxa >5% overall average biomass per red maple (*Acer rubrum*) leaf pack between streams. P-values in boldface are <0.05. Data were log (x+1) transformed. (+) indicates direction of effect in treatment vs. reference stream.

	Before Enrichment		After Enrichment	
Invertebrate Taxon	F	p	F	p
Shredder				
Trichoptera				
Lepidostoma spp.	0.41	0.27	12.41	(+) **0.002**
Pycnopsyche spp.	0.32	0.32	3.62	0.19
Plecoptera				
Tallaperla spp.	0.22	0.86	0.33	0.31
Diptera				
Tipula spp.	0.49	0.23	0.01	0.82
Predator				
Odonata				
Lanthus spp.	9.47	(-) **0.004**	1.02	0.11

Table 2.9. Comparison of relative responses of rhododendron (*Rhododendron maximum*) and red maple (*Acer rubrum*) to nutrient enrichment. Breakdown rates and invertebrate biomass in the treatment stream for both years of enrichment were compared with the average breakdown rates from both streams during the 2 years of pre-treatment. Average values across all dates were compared between streams for respiration. For C:N, comparisons were made between slopes of the regression lines between streams and, due to interannual variability, were compared separately for both years. Boldface indicates a stronger response to enrichment.

	Magnitude of Effect Year 1	Magnitude of Effect Year 2
Breakdown Rate (-*k* day^{-1})		
Rhododendron	1.6	**3.2**
Red maple	**1.7**	2.8
Respiration		
Rhododendron		**3.1**
Red maple		3.0
C:N		
Rhododendron	1.9	**3.1**
Red maple	**2.6**	2.3
Invertebrate Biomass per Leaf Pack		
Rhododendron	**2.3**	2.6
Red maple	1.7	**3.0**
Invertebrate Biomass per g leaf AFDM		
Rhododendron	7.1	**71.6**
Red maple	**10.6**	14.3
Invertebrate Total N : Leaf Total N		
Rhododendron	**6.3**	**44.3**
Red maple	1.8	5.8

Figure 2.1. Mean ln % AFDM leaves remaining ±1SE for rhododendron ((A) Pre-Tmt Yr 1; Ref: y=-0.0033x + 4.67, Tmt: y=-0.0035x + 4.66 (B) Pre-Tmt Yr 2; Ref: y=-0.0064x + 4.73, Tmt: y=-0.0063x + 4.67 (C) N+P Yr 1; Ref: y=-0.0037x + 4.68, Tmt: y=-0.0077x + 4.71 (D) N+P Yr 2; Ref: y=-0.0043 + 4.69, Tmt: y=-0.0157x + 4.74) and red maple ((E) Pre-Tmt Yr 1; Ref: y=-0.01x + 4.65, Tmt: y=-0.0109x + 4.65 (F) Pre-Tmt Yr 2; Ref: y=-0.0098x + 4.53, Tmt: y=-0.0066x + 4.59 (G) N+P Yr 1; Ref: y=-0.0093x + 4.53, Tmt: y=-0.0163x + 4.58 (G) N+P Yr 2; Ref: y=-0.0085x + 4.41, Tmt: y=-0.0262x + 4.41) leaves from the reference (open circles and dashed lines) and treatment (closed circles and solid lines) streams. P-values are from ANCOVA between streams (n.s. = $p>0.05$).

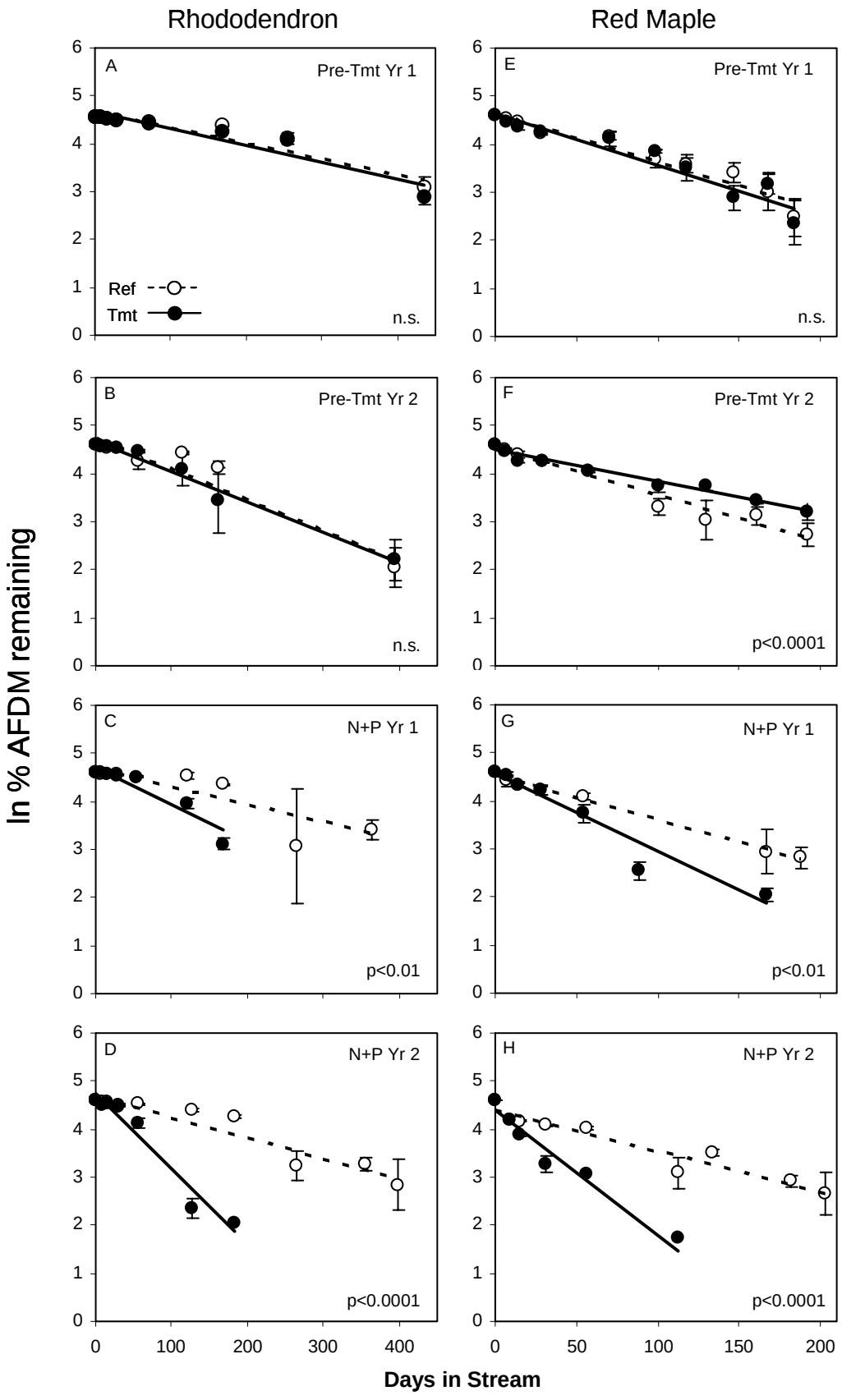

Rhododendron
Red Maple
A
Pre-Tmt Yr 1
Ref
Tmt
n.s.
E
Pre-Tmt Yr 1
n.s.
B
Pre-Tmt Yr 2
n.s.
F
Pre-Tmt Yr 2
p<0.0001
C
N+P Yr 1
p<0.01
G
N+P Yr 1
p<0.01
D
N+P Yr 2
p<0.0001
H
N+P Yr 2
p<0.0001
ln % AFDM remaining
Days in Stream

Figure 2.2. Respiration rates from rhododendron and red maple leaf pack discs during the 2002 season (start date December 5, 2001) from the reference (open circles and dashed lines) and treatment (closed circles and solid lines) streams. For all data points n=3. P-values are from repeated measures MANOVA (ns=non-significant at $p>0.05$).

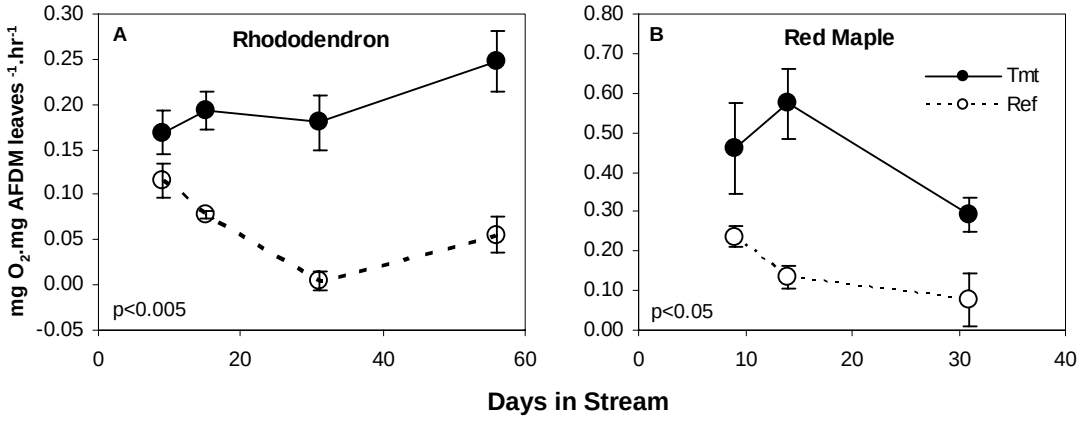

A
Rhododendron
p<0.005
B
Red Maple
p<0.05
Tmt
Ref
mg O2.mg AFDM leaves -1.hr-1
Days in Stream

Figure 2.3. C:N versus number of days since the deployment of leaf packs for rhododendron ((A) Pre-Tmt Yr 2; Ref: y=-0.1673x + 121.7, Tmt: y=-0.2416 + 123.7 (B) N+P Yr 1; Ref: y=-0.03263x + 162.07, Tmt: y=-0.6269 + 171.01 (C) N+P Yr 2; Ref: y=-0.02895x + 153.93, Tmt: y=-0.8824x + 146.68) and red maple (D) Pre-Tmt Yr 2; Ref: y=-0.295x + 103.96, Tmt: y=-0.l316x +96.92 (E) N+P Yr 1; Ref: y=-0.3736x + 115.82, Tmt: y=-0.9519x + 115.51 (F) N+P Yr 2; Ref: y=-0.2255x + 105.02, Tmt: y=-0.5285x + 90.54). The reference stream is indicated by open circles and dashed lines, and the treatment stream is indicated by closed circle and solid lines. All regression lines are significant ($P<0.002$).

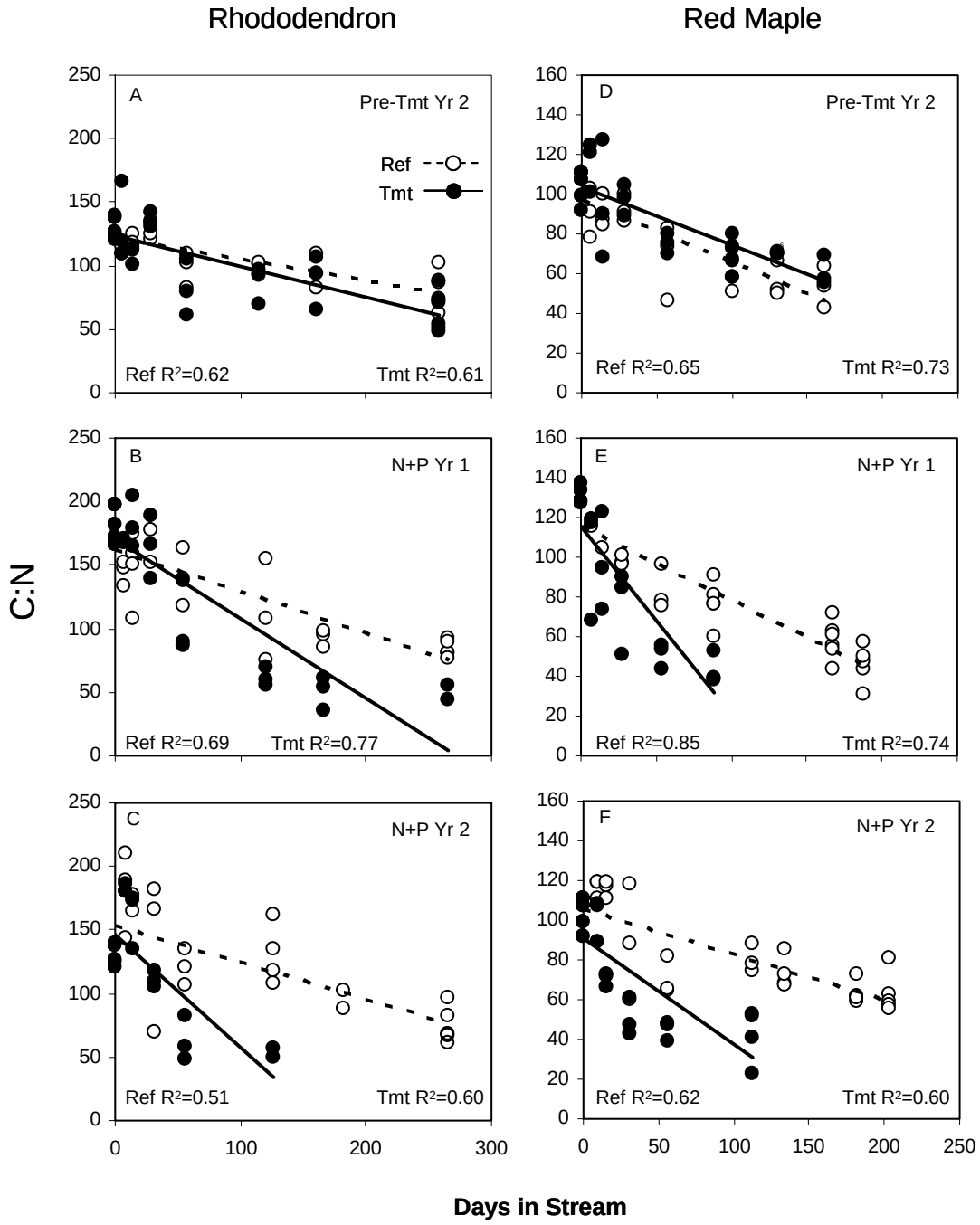

Rhododendron
Red Maple
A
Pre-Tmt Yr 2
Ref
Tmt
Ref R²=0.62
Tmt R²=0.61
D
Pre-Tmt Yr 2
Ref R²=0.65
Tmt R²=0.73
B
N+P Yr 1
Ref R²=0.69
Tmt R²=0.77
E
N+P Yr 1
Ref R²=0.85
Tmt R²=0.74
C
N+P Yr 2
Ref R²=0.51
Tmt R²=0.60
F
N+P Yr 2
Ref R²=0.62
Tmt R²=0.60
C:N
Days in Stream

Figure 2.4. Average invertebrate biomass estimated annually (A, E) and for Day 14 (B, F), Day 55 (C, G) and Day 160 (D, H) in rhododendron leaf packs during the 2 years of pre-treatment and the 2 years of nutrient enrichment as expressed as mg per leaf pack and mg per g leaf AFDM remaining. Arrows indicate start of nutrient enrichment. Error bars represent ±1SE of the total.

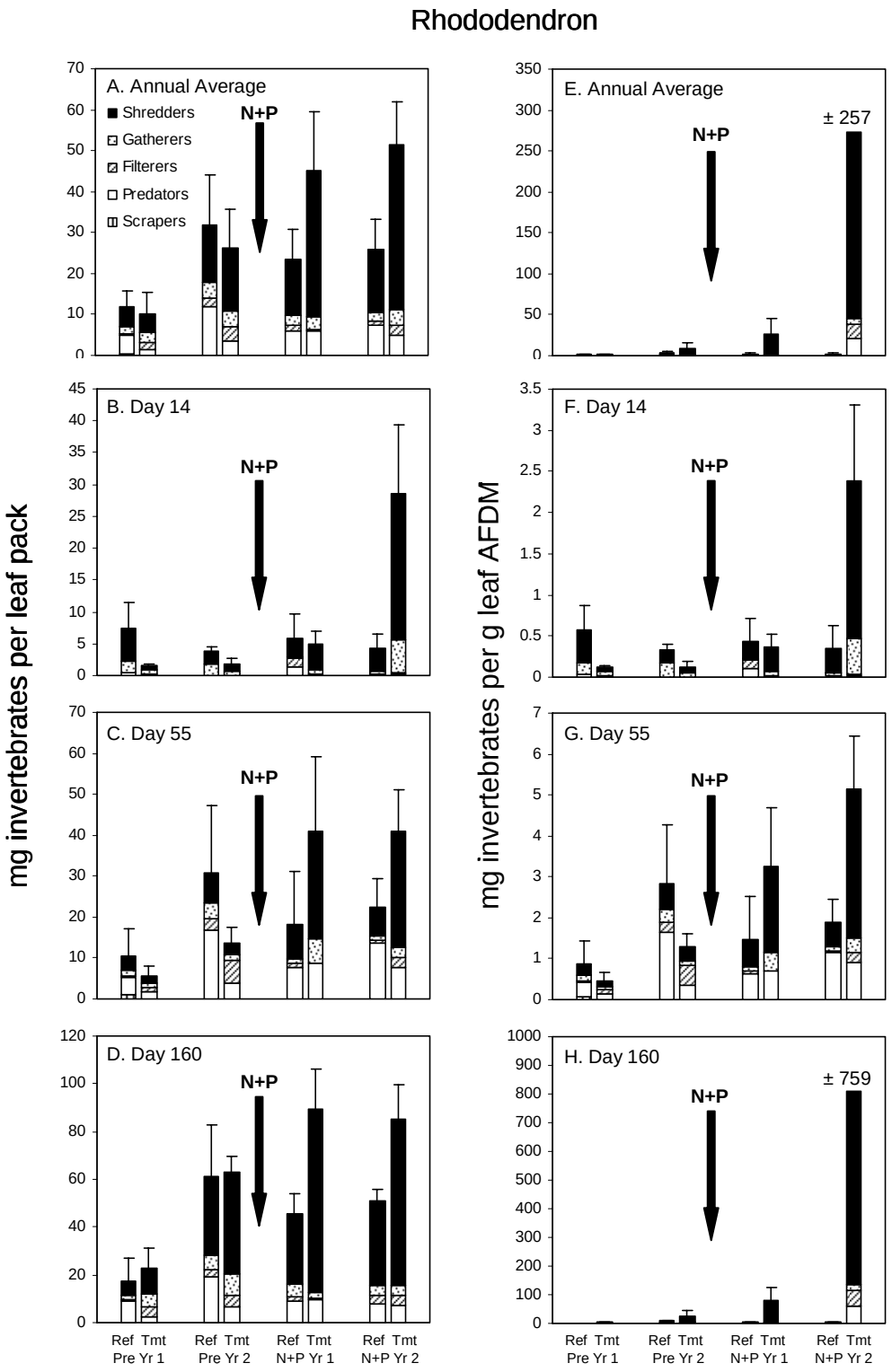

Rhododendron
mg invertebrates per leaf pack
mg invertebrates per g leaf AFDM
A. Annual Average
Shredders
Gatherers
Filterers
Predators
Scrapers
N+P
E. Annual Average
± 257
B. Day 14
F. Day 14
C. Day 55
G. Day 55
D. Day 160
H. Day 160
± 759
Ref Tmt
Pre Yr 1
Pre Yr 2
N+P Yr 1
N+P Yr 2

Figure 2.5. Average invertebrate biomass estimated annually (A, E) and for Day 14 (B, F), Day 55 (C, G) and Day 160 (D, H) in red maple leaf packs during the 2 years of pre-treatment and the 2 years of nutrient enrichment as expressed as mg per leaf pack and mg per g leaf AFDM remaining. Arrows indicate start of nutrient enrichment. Error bars represent ±1SE of the total.

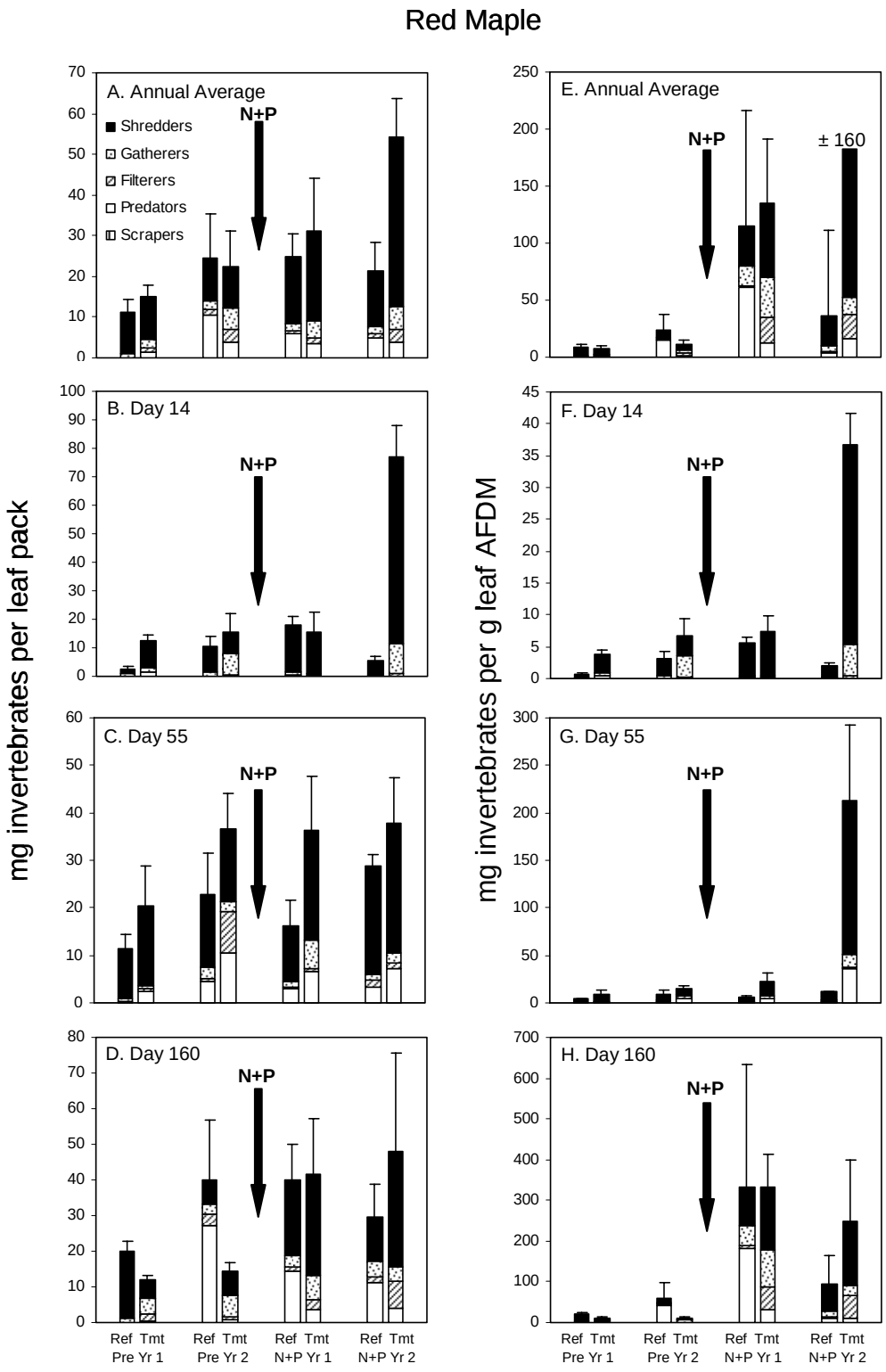
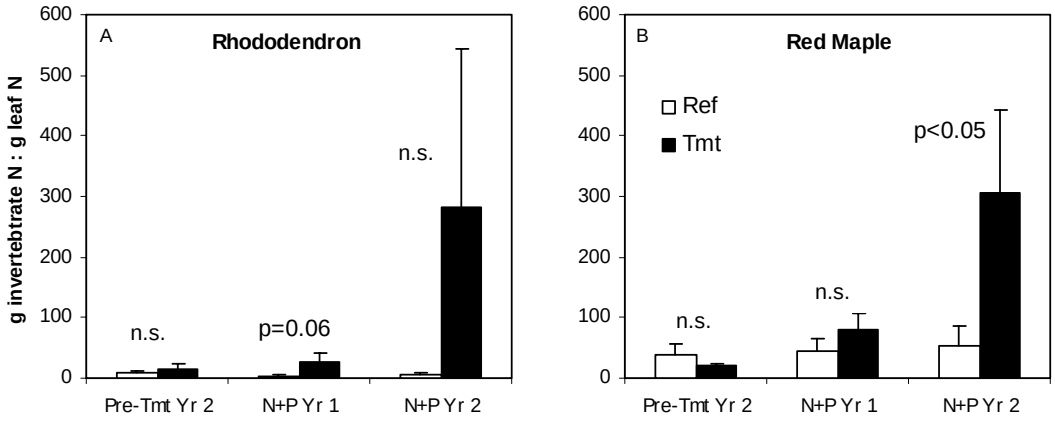

Red Maple
mg invertebrates per leaf pack
mg invertebrates per g leaf AFDM
A. Annual Average
B. Day 14
C. Day 55
D. Day 160
E. Annual Average
F. Day 14
G. Day 55
H. Day 160
N+P
± 160
Shredders
Gatherers
Filterers
Predators
Scrapers
Ref Pre
Tmt Yr 1
Ref Pre
Tmt Yr 2
Ref N+P
Tmt Yr 1
Ref N+P
Tmt Yr 2

Figure 2.5 Average ratio (±1SE) of total nitrogen contained in invertebrate biomass to total nitrogen contained in leaves for leaf packs for the second year of pre-treatment and both years of enrichment. P-values are from a Wilcoxon test between streams for that year (ns=non-significant at $p>0.10$).


A
Rhododendron
g invertebtrate N : g leaf N
600
500
400
300
200
100
0
n.s.
p=0.06
n.s.
Pre-Tmt Yr 2
N+P Yr 1
N+P Yr 2
B
Red Maple
Ref
Tmt
p<0.05
n.s.
n.s.
Pre-Tmt Yr 2
N+P Yr 1
N+P Yr 2

CHAPTER 3

PERIPHYTON RESPONSE TO LONG-TERM NUTRIENT ENRICHMENT IN A SHADED HEADWATER STREAM[1]

[1] Greenwood, J.L. and A.D. Rosemond. Submitted to *Canadian Journal of Fisheries and Aquatic Sciences*.

Abstract

We maintained elevated but moderate concentrations of nitrogen and phosphorus continuously for two years in a heavily shaded headwater stream and compared effects on stream periphyton to a reference stream. Both streams were sampled for one year pre-treatment. Periphyton biomass measured as ash-free dry mass and chlorophyll *a* was significantly higher with enrichment, but the response of chlorophyll *a* was most likely due to higher chlorophyll per cell, with differences being greatest during high light months (Nov. – May). Periphyton total biovolume did not change with enrichment. Cellular growth rates (measured as a proxy for productivity) were stimulated by nutrient enrichment and were much higher in high light vs. low light months. Algal assemblages were dominated by diatoms and remained remarkably similar between the treatment and reference stream throughout the enrichment period. Consistent with short-term experimental work, long term effects of nutrient addition on periphyton were small in magnitude and were potentially suppressed by light availability and invertebrate consumption. These and other factors may have also been important in defining the limited algal species pool in these streams that could potentially respond to enrichment. Our results indicate that in headwater streams with intact tree canopies, chronic nutrient enrichment at moderate concentrations may have little detectable effect on periphyton composition or biomass.

Introduction

Rivers play critical roles on the landscape in providing essential services to humans. Intact headwater streams are crucial to the functioning of river systems (Meyer and Wallace 2001) and have been shown to be critical sites in river networks for processes such as nutrient uptake and retention (Peterson et al. 2001). However, headwater streams are also particularly

vulnerable to changing land use and non-point source pollutants. In many cases, these streams are not protected simply because they do not appear on maps or are not adequately protected by law (Meyer and Wallace 2001). Small order streams also have relative greater contribution of watershed area to stream area compared to larger streams (Selby 1985). Thus, first-order streams may experience greater nutrient inputs than larger streams due to atmospheric deposition (via saturation of terrestrial ecosystems or mobilization from soils from the surrounding catchment). However, the effects of long-term nutrient input to forested headwater streams are essentially unknown.

Most experimental work examining effects of enrichment on periphyton has been short-term in nature (ca. < 8 weeks; Francoeur 2001) and thus demonstrates potential or transient response, rather than ultimate effects. The importance of long-term studies is evident from a nearly 20 year enrichment of the Kuparak River in the Alaskan tundra, where enrichment resulted in profound effects on algal biomass that were observed during the first two years of enrichment but were suppressed by grazers in two subsequent years (Miller et al. 1992; Peterson et al. 1993). Further, a dramatic increase in bryophyte cover reduced the magnitude of enrichment effects on periphytic biomass (Slavik et al. 2004). However, the high light environment of a tundra stream like the Kuparak contrasts with many headwater streams that are heavily shaded. Short-term experimental work in forested headwater streams indicates that the response of algal biomass to nutrient enrichment is limited by ambient light levels (Gregory 1980; Triska et al. 1983; Lowe et al. 1986; Hill and Knight 1988; Winterbourn 1990; Rosemond 1993; Wellnitz et al. 1996; Rosemond et al. 2000; Hill et al. 2001; Mosisch et al. 2001; Tank and Dodds 2003; Bernhardt and Likens 2004). In environments where response to enrichment is predicted to be subtle or suppressed by other factors (systems with heavy shade or where algal

biomass is controlled by grazers), longer-term studies may be needed to predict the potential ultimate effects of nutrients on periphyton structure and function.

Previous work (Miller et al. 1992; Peterson et al. 1993) in well-lit streams has suggested that algal biomass accrual due to long-term enrichment can be controlled by top-down consumption. Less predictable is whether periphyton assemblage composition will change in response to enrichment. Algal assemblages are often sensitive to changes in water chemistry due to different tolerance optima among individual populations (Lowe 1974) or changes in competitive ability among species (Tilman 1977). The great majority of studies that have examined species assemblage response to elevated nutrient concentrations have found significant shifts in taxonomic composition (e.g. Fairchild et al. 1985; Marks and Lowe 1989; Burton et al. 1991; Mulholland and Rosemond 1992). However, community shifts are difficult to predict since they do not always change with nutrient enrichment, regardless of the biomass response (Lohman et al. 1991; Peterson et al. 1993; Shortreed et al. 1984). Specifically, in some situations other factors (i.e. stressful or poor growing conditions, intense grazing) can be more important than nutrients in driving variation in assemblage composition and can minimize the capacity of species assemblages to respond to nutrient enrichment (Hill et al. 1992; Rosemond et al. 2000).

Our study examined periphyton response to long-term nutrient addition in a shaded, headwater stream. We determined the effects of a 2-year continuous enrichment of nitrogen and phosphorus on several characteristics of epilithic periphyton assemblages. We measured algal biomass as ash-free dry mass (AFDM) and chlorophyll *a*, determined algal species composition, and assessed algal growth rates as a proxy measure of productivity. We hypothesized that due to light limitation, nutrient enrichment would have little overall effect on algal biomass, and cellular growth rates of periphyton would be constrained. However, because of variation in

nutrient optima among algal taxa, we predicted that periphyton assemblage structure would be altered via changes in the relative abundance of common species.

Methods

Study Site

Two headwater streams, one enriched and one serving as a reference were examined for 3 years (July 1999 – July 2002) at the Coweeta Hydrologic Laboratory (CHL), a USDA Forest Service research facility located in the Blue Ridge Mountain physiographic province in the southern Appalachian mountains (North Carolina, U.S.A.). The reference stream (Catchment 53) and the enriched stream (Catchment 54) have similar physical and chemical characteristics (Table 3.1). Dominant vegetation includes tulip poplar (*Liriodendron tulipifera* L.), white oak (*Quercus alba*, L.), red oak (*Quercus rubra*, L.), red maple (*Acer rubrum*, L.), and dogwood (*Cornus florida*, L.) (Swank and Crossley 1988). Rhododendron (*Rhododendron maximum* L.), an evergreen, grows as a dense understory in the riparian zone. Thus, a "double canopy" of deciduous trees and rhododendron shades the study streams to some degree during all seasons.

Nutrient Enrichment

Pretreatment data were collected from both streams from July 1999-July 2000. Beginning 11 July 2000, the treatment stream was continuously enriched with NH_4NO_3, KH_2PO_4 and K_2HPO_4 along the entire 150m length of the study reach for two years. A dissolved nutrient salt solution was pumped into an irrigation line (approx. 2cm diameter) that was fed with stream water from an upstream head tank. The line ran the entire length of study reach adjacent to the streambed with nutrient solution delivered from multiple spigots. A metering pump (Liquid Metronics, Inc.) was electronically linked with a Campbell data logger to an ISCO flow

measurement device at the downstream end of the stream reach to deliver nutrients in a discharge-dependent manner. Stream water nutrient concentrations were measured during the pretreatment and experimental periods. During the pretreatment period, one to four samples were taken from the reference and treatment streams on five sampling dates in the reference stream and 12 sampling dates in the treatment stream. During the enrichment period, one sample was taken from the reference stream and five samples were taken along the length of the treatment stream approximately every two weeks to confirm that nutrients were elevated in an even distribution within the study reach. Stream water samples were filtered with Millipore HA filters into acid-washed bottles and frozen until analysis. Concentrations of NO_2^--N, NO_3^--N, NH_4^+-N and soluble reactive phosphorus (SRP) were determined with an Alpkem Rapid Flow Analyzer 300 at the University of Georgia Chemical Analysis Laboratory.

Biomass

Epilithic algae were sampled from unglazed ceramic tiles (5.3 cm^2) every two months on approximately the 15^{th} of the month from July 1999 through July 2002. Tiles were colonized for two months prior to collection (i.e. when tiles were collected they were replaced with uncolonized tiles that were collected 2 months later). Five sets of two tiles were secured to the streambed along the length of each stream. Accumulated debris (particularly allochthonous leaf material) was cleared from tiles at least once per week. One tile was used for determination of AFDM and the other for chlorophyll *a*. Periphyton used for AFDM samples was brushed from the tile with a toothbrush, rinsed, and the resulting slurry filtered onto a pre-ashed Gelman A/E glass fiber filter. The filters were weighed before and after combusting at 500°C to determine AFDM. Chlorophyll *a* was measured by extracting colonized tiles directly in 20mL of 90% alkaline (with NH_4OH) acetone solution in a freezer for 24 hours. Chlorophyll *a* content was

measured with a Turner Model 112 (July 1999 - May 2001) and a Turner TD-700 (July 2001-July 2002) fluorometer. Photosynthetically active radiation (PAR) was measured starting July 1999 in the reference stream and November 1999 at the treatment stream at each sampling during the study period with a Li-Cor Model LI-250 hand-held light meter.

Growth Rates

We sought to determine a measure of algal productive capacity between the two streams that was not influenced by potential losses due to grazers and which also examined potential effects of irradiance on nutrient response. Algal productivity in these streams was too low to measure with an oxygen change method and measuring ^{14}C uptake was logistically problematic. Thus, we used periphyton cellular growth rates as a proxy for productive capacity by assessing accrual rates of algal cells on glass slides before and after canopy closure. This method also allowed us to measure periphyton accrual in the absence of grazers, which were excluded from experimental chambers. In-stream channels were deployed for 4 weeks during 4 separate trials in "March" (21 Mar-4 Apr), "April" (18 Apr-1 May), "June" (21 May-5 Jun) and "July" (26 Jun-10 Jul) 2002. In-stream channels were constructed using 30cm lengths of vinyl rain gutter. To exclude grazers, two layers of 200μm Nitex mesh were glued with silicon sealant to both ends of the gutter. No grazers were detected in the gutters during the experiment. Five gutters each were placed in the treatment and reference streams. Microscope slides were used as the colonization substratum for periphyton. Three slides were glued horizontally into each channel with silicon sealant. One slide was collected after two weeks (two weeks of accrual time) and another slide at four weeks (total of four weeks of accrual time) from each of the five gutters in each stream. Slides were scraped with a razor blade and fixed in 1ml of 2.5% formalin. Cell densities were enumerated in a Palmer-Maloney cell as for species composition. New cell accrual between

weeks two and four was calculated as the increase in number of cells per day which was compared between each stream for each date. This time frame was adequate for distinguishing growth period (2-4 week accrual) from confounding factors of the colonization period (0-2 week accrual) and avoided the potential for longer-term biomass accrual obscuring differences in growth rates.

Species Composition and Biovolume

Beginning in September 1999, one additional tile was collected with each set of biomass tiles for periphyton taxonomic analysis. Tiles were brushed with a toothbrush, rinsed, and the entire slurry fixed in a total of 20 mL solution of 2.5% formalin. Community composition and cell density were determined by counting at least 500 cells in a Palmer-Maloney nannoplankton counting chamber at 400X. In cases where cell densities were too low to find 500 cells, 10 transects of the diatom slide were examined. Diatom species were identified after cleaning with 30% H_2O_2 and $K_2Cr_2O_7$ to clear cell contents and permanently mounting in Naphrax high resolution mounting medium. Species composition was assessed by identifying 300 complete frustules at 1000X. In cases where cell densities were too low to find 300 frustules, 10 transects of the diatom slide were examined and all complete frustules found were identified. To calculate algal cell biovolume, average dimensions of up to 10 cells in each taxon were applied to standard geometric shapes that best represented the shape of each taxon (Hillebrand et al. 1999). Biovolumes from the Academy of Natural Sciences database from the Phycology Section of the Patrick Center for Environmental Research biovolume database (http://diatom.acnatsci.org/autecology/#browse) were used to estimate biovolumes for rarely encountered taxa.

Data analysis

Randomized intervention analysis (Carpenter et al. 1989), or RIA, was used to assess differences between the reference and treatment streams in AFDM, chlorophyll *a*, total biovolume and relative biovolume of algal species with >5% average biovolume from tiles. RIA detects differences between variables in a time series between unreplicated reference and treatment systems. Kruskall-Wallis and Student's t multiple comparison tests were used to compare seasonal levels of log-transformed PAR and biomass data. Changes in algal growth rates between streams were assessed with *t*-tests.

Results

Nutrient Enrichment

The enrichment increased DIN ~13X and SRP ~5X to approximately 400µg/L and 50 µg/L SRP in the treatment stream (Table 3.2), resulting in a stream water molar N:P ratio of about 18:1. Stock solution was added at a molar N:P ratio of approximately 11:1, which suggests that there was a relatively greater uptake of P. These nutrient concentrations are within range of those found regionally in streams (Scott et al. 2003). Nutrient levels in the reference stream remained consistent across pretreatment and treatment periods with DIN <30µg/L and SRP <10µg/L.

Biomass response

Periphyton biomass, as both AFDM (Fig. 3.1A) and chlorophyll *a* (Fig. 3.1B) were both significantly greater in the treatment relative to reference stream after nutrient enrichment (RIA, $p<0.05$). Chlorophyll *a* was typically 2-9 mg m^{-2} prior to enrichment, and increased to 23 mg m^{-2} in the treatment stream in Year 1 (in May) of enrichment but only up to 17 mg m^{-2} in Year 2 (in

March). Pretreatment values for AFDM were low and similar in the two streams (100-300 mg m^{-2}) and although both streams were higher in the period after July 2000, AFDM was roughly 50% greater in the treatment vs. reference stream beginning in March 2001 (up to 900 mg m^{-2}). Chlorophyll *a* showed a greater increase during the spring of each year compared to summer and fall. Increased AFDM in the treatment stream showed no such seasonal effect. There was very little difference in either AFDM or chlorophyll *a* between treatment and reference streams on our last two sampling dates, in summer 2002.

Periphyton biomass as total biovolume (Fig. 3.2A) was not significantly different between streams after nutrient enrichment (RIA $p>0.05$). Periphyton exhibited a slight seasonal pattern where biovolume was lowest, less than $2x10^6$ μm^3 cm^{-2}, during November or January, and reached a slightly higher maximum, up to about $4x10^6$ μm^3 cm^{-2} in mid- to late summer. An extremely high value for biovolume in the reference stream after enrichment was associated with one replicate having unusually high algal accrual. The ratio of chlorophyll *a* to biovolume (Fig 2B) contained a very high value in the reference stream prior to enrichment due to a very low chlorophyll *a* value (0.1×10^6). This data point was considered spurious and was removed before RIA. Thus, there was also no significant difference between streams in the ratio of chlorophyll *a* to biovolume (Fig. 3.2B, RIA, $p>0.05$). However, there was a trend of higher values during the spring months, as was seen for chlorophyll *a*.

Relationships between periphyton biomass and irradiance

To determine whether variation in periphyton response to nutrients was due to season, we compared average light levels during periods of high (Jan, Mar, May, Nov) and low (Jul, Sep) light and then compared algal biomass between treatment and control streams during these time periods. Regressions run between biomass measurements and light levels with and without

enrichment were nonsignificant ($p>0.05$) with little variation explained by the model ($r^2 < 0.05$), most likely due to the variability inherent in instantaneous light readings. Thus, we decided to use longer-term averages to assess the relationship between seasonal light and chlorophyll *a* levels. Data from the treatment stream during pre-treatment was included with values for the reference stream. Light data were lost for July 2000 and January 2001 in the treatment stream due to instrument malfunction. Instantaneous PAR levels were low year round, averaging about 150 μmol m^{-2} sec^{-1} Nov-May and 10 μmol m^{-2} sec^{-1} Jul-Sep (Fig. 3.3A). Light levels were significantly higher during Nov-May in both streams compared to July-Sep. (Kruskall-Wallis $H=19.16$, $df=3$, $p<0.05$; Student's t multiple comparison $p<0.05$). Likewise, chlorophyll *a* levels were signficantly higher in the treatment vs. reference stream in Nov-May but were not different between streams for the period Jul-Sep (Fig. 3.3B, Kruskall-Wallis $H=7.85$, $df=3$, $p<0.05$; Student's t multiple comparison $p<0.05$). AFDM levels were significantly higher in the treatment versus the reference stream, regardless of time of year (Fig. 3.3C, Kruskall-Wallis $H=11.22$, $df=3$, $p<0.005$, Student's t multiple comparison $p<0.05$). Biovolume was significantly higher only in the reference stream between high light and low light times of year (Fig. 3.3D, Kruskall-Wallis $H=13.71$, $df=3$, $p<0.005$, Student's t multiple comparison $p<0.05$). This difference was likely due to the unusually high biovolume measured in the reference stream in September 2001.

Growth Rates

Accrual rates during March and April (Fig. 3.4A) were not significantly different between streams due to higher variability, but the range of the accrual rates were much higher in the treatment stream. Out of the four months that trials were run, cell accrual rates were significantly different between streams only during June (Fig 4B; *t*-test $p<0.05$, $t= -2.4$, $df=8$) and July (Fig 4B; *t*-test $p<0.05$, $t= -2.3$, $df=8$). Also, the range in accrual rates in the treatment

stream decreased by an order of magnitude from April to June and again by an order of magnitude from June to July.

Species Composition

Diatom taxa were dominant (>98% of algal biovolume on average) in both the reference and treatment streams throughout the experiment (Table 3.3). Of the 35 taxa identified, most were rare with five species making up nearly 95% of the biovolume in both streams (Table 3.4). None of these taxa showed significant differences in biovolume between the reference and enriched streams (Fig. 3.5, RIA $p>0.05$). By far the dominant taxa in the streams were *Euntoia pectinalis* var. *minor* and *Meridion constrictum*, which exhibited somewhat predictable seasonal trends. Biovolume of *Eunotia pectinalis* var. *minor* was low in the spring months and peaked in the late summer and fall (Fig. 3.5A), whereas biovolume of *Meridion constrictum* was greatest in spring, and declined through the summer months (Fig. 3.5B). Although important contributors to total biovolume, *Eunotia pectinalis* var. *recta* (Fig 3.5C) and *Gomphonema parvulum* (Fig. 3.5D) showed no strong seasonal patterns or treatment effects. *Navicula tantula* showed what was potentially a delayed response to enrichment, by increasing in relative biovolume from about 10% to nearly 50% in the enriched stream during the last 2 sampling dates (Fig. 3.5E).

Discussion

Effects of nutrient addition on stream periphyton

Our 2-yr nutrient enrichment of a first-order forested stream resulted in very little sustained change in stream periphyton. Different aspects of the periphyton responded differently to nutrient enrichment, which together, illustrate the effects that chronic nutrient enrichment may have in similar headwater streams. Biomass response of primary producers was either seasonally

transient (chlorophyll *a*) or did not occur (algal biovolume). Changes in chlorophyll *a* appeared to be largely driven by a physiological response of cellular increases in chlorophyll, rather than a proliferation of cells. Algal cellular growth rates were stimulated by nutrient addition, but the magnitude of the response was quite small under seasonally low light levels, suggesting constraints due to light availability. Only biomass measured as ash-free dry mass, which includes heterotrophic components of periphyton, was consistently positively affected by nutrient enrichment. Short term experimental studies have shown that a transient response to nutrient enrichment can be quite high in the absence of grazers and under high light conditions (e.g. Rosemond 1993; Hillebrand 2002), whereas such responses are small when top-down consumption or other limiting factors also constrain algal biomass (Lowe et al. 1986; Steinman et al. 1989; Hart and Robinson 1990; Hill et al. 1992; Miller et al. 1992; Peterson et al. 1993; Rosemond et al. 1993; Mosisch et al. 2001). Results from our long term study in which primary producer response was either seasonally dependent or apparently controlled by other factors are consistent with such studies.

Irradiance was likely an important factor in the expressed response of periphyton to nutrient enrichment. Seasonal variation in chlorophyll *a*, the amount of chlorophyll *a* per cell, and measures of algal cell growth pre- and post-canopy closure, suggest that irradiance strongly influenced all of these variables. Seasonal variation in chlorophyll *a* appeared to be at least partly due to a trend of increased chlorophyll *a* produced per cellular unit rather than an increase in biovolume or cell densities. In previous studies, increased chlorophyll *a* per cell in periphyton has occurred in response to increased nutrients (Rosen and Lowe 1984; Geider et al. 1993, Rosemond 1993). Interestingly, the nutrient-driven response of higher chlorophyll *a* per cell under high light conditions we observed is opposite to the predicted response (in the absence of

enrichment) in which taxa with greater relative chlorophyll *a* per cell or a physiological shift to greater chlorophyll *a* per cell is observed in response to low light environments (e.g., Rosemond 1993, Felip and Catalan 2000). Contributing to this pattern was the fact that total biovolume was temporally variable, was not affected by enrichment, and showed a trend to be higher in low vs. high light months, whereas chlorophyll *a* exhibited a response to nutrients in only high light months. This suggests that physiological changes and the manufacturing of the N-containing chlorophyll molecule were limited by nutrient availability but were not accompanied by biomass changes.

Changes in stoichiometry may signal other physiological changes in periphyton in response to enrichment. Stoichiometric changes in epilithon were also observed in which both C:N and C:P of epilithic scrapings in the treatment stream were greatly reduced compared to the reference stream (Cross et al. 2003). However, since these nutrient concentrations were measured from the entire epilithic community, it can not be determined if the source of change was from autotrophic, heterotrophic or both components of the periphyton.

Our data indicate that heterotrophic compared to autotrophic components of periphyton biomass responded more consistently to nutrient enrichment. Ash-free dry mass, which is a measure of periphyton biomass that likely includes heterotrophic components (e.g., fungi, bacteria and trapped organic particles), increased in response to enrichment, but quantification of the strictly algal response, algal biovolume, did not. Heterotrophic microorganisms associated with leaves (Gulis and Suberkropp 2003) and wood (Gulis et al. in press) increased dramatically in the treatment stream during this study, indicating that positive effects of nutrients on heterotrophs living on other substrates (e.g., tiles) would be likely. However, autotrophs may

also have contributed to variation in AFDM via the production of extracellular organic material by algae which could potentially increase in response to enrichment (Hoagland et al. 1993).

How much of a lack of periphyton response could be attributed to herbivore consumption vs. light availability?

The seasonal pattern in algal growth rates and chlorophyll *a* was most likely the result of light availability in these headwater streams. The maximum response observed in chlorophyll *a* and growth rates, the only definitive responses to enrichment, occurred when light levels reaching the stream were high. Also, data from whole-stream metabolism measurements (P.J. Mulholland, unpublished data) showed a higher rate of gross primary production (GPP) with enrichment during the spring months when light levels are higher, although overall GPP was always very low (<10 mg O_2 m^{-2} day^{-1}). Other studies have shown that periphyton productivity can be primarily limited by light (Hill et al. 1995; Hill and Knight 1988; Hill et al. 2001) and that the importance of light, relative to other limiting factors, can change seasonally (Rosemond et al. 2000). Rates of photosynthesis generally reach a maximum at light levels between 100 and 200 μmol m^{-2} s^{-1} in forested streams (Hill et al. 1995). Instantaneous irradiance levels during spring and fall often reached or exceeded 200 μmol m^{-2} s^{-1} in both streams, indicating that light limitation was most likely less important during this time than during the summer when irradiance levels were less than 20 μmol m^{-2} s^{-1}.

Although there was no response in autotrophic biomass, productivity of algal assemblages, as cellular growth rates, was stimulated by nutrient addition. Although tests of these effects were only significant in the two months with closed canopy (June, July), differences between high and low nutrient streams were greatest in magnitude prior to canopy closure (March, April) during seasonally high light levels. It is possible that consumption by invertebrate

grazers precluded such increased productivity from resulting in higher periphyton biomass in the study stream. Specifically, despite observed nutrient-driven increases in algal growth rates that were fairly large in magnitude, basal levels of algal biomass in these streams are so low that invertebrate grazers can likely easily consume any accumulated biomass. As a potential indication of herbivores tracking and controlling algal resources in these streams, we examined whether invertebrate scraper biomass was similarly higher in higher light months, as we had observed with chlorophyll *a*. Over the time period of enrichment, mean monthly scraper biomass was low in the period June-October (n = 11 per stream): reference stream 23.1 mg m^{-2} (3.4 S.E.), treatment stream 26.2 mg m^{-2} (8.6 S.E.) and was higher in the higher light period (n = 14 per stream): reference stream 41.4 mg m^{-2} (8.7 S.E.), treatment 91.2 mg m^{-2} (29.6 S.E.). The greatest scraper biomass was observed in the treatment stream during the high light period, but overall differences between time periods and streams were not significant (W.F. Cross, unpublished data). Short-term studies have shown nutrient stimulation of algal productivity and growth of invertebrates in systems where invertebrate grazers control periphyton biomass (e.g. Hart and Robinson 1990; Rosemond et al. 1993; Steinman 1992). Further, the increase in scrapers provides support for the possibility that autotrophic components of periphyton were under dual control by nutrients and light levels, since potential grazer response to nutrient addition was only during the spring months.

Although cellular growth rates and chlorophyll *a* concentrations were higher in the treatment stream during periods of high light, there was no response in biomass as measured by algal biovolume. Periphyton biomass is typically low in headwater streams with intact forest canopies. Compared to data complied by Dodds et al. (1998) on chlorophyll *a* levels for over 200 temperate streams, the chlorophyll *a* values that we measured were in the 10^{th} percentile in the

reference stream, and the 20th percentile with enrichment for both mean and maximum measures of chlorophyll *a*. Previous work has also shown that in forested headwater streams, periphyton biomass is constrained by multiple factors and that biomass response to nutrient manipulations is typically negligible due to other limiting factors (Bernhardt and Likens 2004; Hill et al. 2001; Rosemond et al. 2000). The fact that we observed significant effects of enrichment on cellular growth rates in the absence of potential consumers and did not see effects of enrichment on periphyton biovolume strongly suggests at least some overriding control of invertebrate consumers on a potential biomass response.

Lack of assemblage composition response

There was remarkable constancy in the composition of these periphyton assemblages in both study streams despite long-term changes in the availability of nutrients in the treatment stream. There were actually more pronounced seasonal effects in algal assemblages compared to nutrient effects, suggesting that light availability was driving the species distribution in this study. *Eunotia pectinalis* var. *minor* and *Meridion constrictum* seemed to trade off dominance in the community, with *E. pectinalis* var. *minor* making up most of the biovolume when light availability was seasonally low and *M. constrictum* dominating during periods of high light. *E. pectinalis* var. *minor* also exhibited preference for low light levels in another deciduous forest stream study (Rosemond 1993) and *M. circulare*, a taxon related to *M. constrictum*, was shown to be stimulated by higher light levels (Rosemond et al. 2000), consistent with seasonal patterns observed in this study. However, *Meridion* has been shown to prefer colder temperatures (Patrick 1971; Lowe 1974) which may explain its peak during the colder months of the year. Toward the end of the study period, two taxa, *G. parvulum* and *N. tantula*, were potentially responding to enrichment, indicating that possibly the algal assemblage was beginning to change toward the

end of the experiment. The increase in *G. parvulum* occurred during the high light months, and in another study at Coweeta, was more common in a clearcut stream (Lowe et al. 1986), suggesting a preference for higher light levels. The reasons for the increase in *N. tantula*, a small diatom common in highly oxygenated environments (Dayner and Johansen 1991), are unclear.

The species pool found on tiles in these headwater streams was largely limited to diatoms, with little presence of chlorophytes or cyanobacteria. Whatever factors restrict taxonomic distribution in our samples may have ultimately constrained any response we observed of periphyton to nutrient enrichment. Chlorophytes might be expected more during seasonal periods of high light, but these taxa were rarely present in our samples year-round. However, chlorophytes increased with enrichment in a near-by stream at Coweeta that had been logged (Lowe et al. 1986). Cyanobacteria have been known to outcompete diatoms in low light situations (Stevenson et al. 1985), and could be expected to thrive during nutrient enrichment, but cyanobacteria were rarely encountered in our tile samples. An analysis of algal communities from different substrata (moss, liverwort, and bedrock) indicates that communities from tiles were largely representative of periphyton communities on natural substrates (J. Greenwood, unpubl. data). About 80% of the taxa found on bedrock were also found on tiles (the other 20% dominated by cyanobacterial filaments), but dominant taxa from tiles only comprised 5-65% of the biovolume on bedrock. Also, algal communities from moss, liverwort and bedrock showed no consistent change in assemblages due to nutrient enrichment (J. Greenwood, unpubl. data), suggesting that regardless of substrata, response of the algal community to nutrient enrichment was minimal.

Our results suggest that assemblages in these streams were mostly limited by factors other than nutrients. Other studies have found very little change in algal species composition in

response to nutrients under conditions of intense grazing (assemblage composition was driven by grazer-resistance more than by response to nutrients; e.g. (Hill et al. 1992; Rosemond et al. 1993). However, the dominant taxa in our study streams would not necessarily be characterized as a flora indicative of a heavily-grazed system (e.g., some taxa are stalked and produce upright cells, in contrast to dominance by prostrate adnate taxa that are grazer resistant). Physical or chemical characteristics (overall low irradiance levels, high turbulence, softwater chemistry) of these streams may also have overriding controls on potential assemblage composition. Regardless of which other controlling factors were most important, subtle physiological changes such as increased chlorophyll *a* or nutrient content, did not translate into enough differential activity among different algal species to result in any shifts in species composition.

Two years of continuous moderate nutrient enrichment resulted in neither shifts in periphyton assemblage composition nor in sustained effects on algal biomass. This limited response can probably be attributed to the low initial standing crop of periphyton, other factors controlling biomass and productivity, and to a limited taxonomic pool of potential responders to nutrient addition. In contrast to nutrient effects on primary producers, analyses of heterotrophic microbial response in these study streams has revealed dramatic effects nutrient addition on bacteria and fungi associated with leaves and wood (Gulis and Suberkropp 2003; Gulis et al. in press; K. Suberkropp, unpublished data). Although we have evidence that shading and potentially invertebrate consumption contributed to the limited response of periphyton, other factors that ultimately limited the algal species pool are unknown but are likely associated with the physiochemistry of undisturbed shaded headwater streams. Our data show that in such streams, periphyton will respond very little to moderate levels of long-term nutrient enrichment.

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Literature Cited

Bernhardt, E.S. and G.E. Likens. 2004. Controls on periphyton biomass in heterotrophic streams. Freshwater Biology 49: 14-27.

Burton, T.M., M.P. Oemke and J.M. Molloy. 1991. Contrasting effects of nitrogen and phosphorus additions on epilithic algae in hardwater and a softwater stream in Northern Michigan. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 24: 1644-1653.

Carpenter, S.R., T.M. Frost, D. Heisey and T.K. Kratz. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology 70: 1142-1152.

Cross, W.F., J.P. Benstead, A.D. Rosemond and J.B. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6: 721-732.

Dayner, D.M. and J.R. Johansen. 1991. Observations on the Algal Flora of Seneca Cavern, Seneca County, Ohio. Ohio Journal of Science. 91: 118-121.

Dodds, W.K., J.R. Jones and E.B.Welch. 1998. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. Water Research 32:1255-1462.

Fairchild, G.W., R.L. Lowe and W.B. Richardson. 1985. Algal periphyton growth on nutrient-diffusing substrates: an *in situ* bioassay. Ecology 66: 465-472.

Felip, M. and J. Catalan. 2000. The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima. Journal of Plankton Research 22:91-106.

Francoeur, S.N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society 20: 358-368.

Geider, R.J., J. La Roche, R.M. Greene and M. Olaizola. 1993. Response of the photosynthetic apparatus of *Phaeodactylum tricornutum* (Bacillariophyceae) to nitrate, phosphate, or iron starvation. Journal of Phycology 29: 755-766.

Gregory, S.V. 1980. Effects of Light, Nutrients, and Grazing on Periphyton Communities in Streams. Ph.D. Thesis. Oregon State University, Corvallis, Oreg.

Gulis, V. and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48: 123-134.

Hart, D.D. and C.T. Robinson. 1990. Resource limitation in a stream community: phosphorus enrichment effects on periphyton and grazers. Ecology 71: 1494-1502.

Hill, W.R., H.L. Boston and A.D. Steinman. 1992. Grazers and nutrients simultaneously limit lotic primary productivity. Canadian Journal of Fisheries and Aquatic Sciences 49: 504-512.

Hill, W.R. and A.W. Knight. 1988. Nutrient and light limitation of algae in two northern California streams. Journal of Phycology 24: 125-132.

Hill, W.R., P.J. Mulholland and E.R. Marzolf. 2001. Stream ecosystem responses to forest leaf emergence in spring. Ecology 82: 2306-2319.

Hill, W.R., M.G. Ryon and E.M. Schilling. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. Ecology 76: 1297-1309.

Hillebrand, H. 2002. Top-down versus bottom-up control of autorophic biomass — a meta-analysis on experiments with periphyton. Journal of the North American Benthological Society 21:349-369.

Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollingher and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403-424.

Hoagland, K.D., J.R. Rosowski, M.R. Gretz and S.C. Roemer. 1993. Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. Journal of Phycology 29: 537-566.

Lohman, K., J.R. Jones and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. Journal of the North American Benthological Society 10: 14-23.

Lowe, R.L. 1974. Environmental Requirements and Pollution Tolerance of Freshwater Diatoms. USEPA, Cincinnati, Ohio.

Lowe, R.L., S.W. Golladay and J.R. Webster. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. Journal of the North American Benthological Society 5: 221-229.

Marks, J.C. and R.L. Lowe. 1989. The independent and interactive effects of snail grazing and nutrient enrichment on structuring periphyton communities. Hydrobiologia 185: 9-17.

Meyer, J.L. and J.B. Wallace. 2001. Lost Linkages and Lotic Ecology: Rediscovering Small Streams. *In* Ecology: Achievement and Challenge. *Edited by* Huntly, N.J. and S. Levin. Blackwell Science, Oxford. pp. 295-317.

Miller, M.C., P. DeOliveira and G.G. Gibeau. 1992. Epilithic diatom community response to years of PO_4 fertilization: Kuparuk River, Alaska (68 N Lat.). Hydrobiologia 240: 103-119.

Mosisch, T.D., Bunn, S.E. and P.M Davies. 2001. The relative importance of shading and nutrients on algal production in subtropical streams. Freshwater Biology 46: 1269-1278.

Mulholland, P.J. and A.D. Rosemond. 1992. Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream-downstream linkage. Journal of the North American Benthological Society 11: 405-419.

Patrick, R. 1971. The effects of increasing light and temperature on the structure of diatom communities. Limnology and Oceanography 16: 405-421.

Peterson, B.J., L. Deegan, J. Helfrich, J.E. Hobbie, M. Hullar, B. Moller, T.E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M.A. Lock, D.M. Fiebig, V. McKinely, M.C. Miller, J.R. Vestal, R. Ventullo and G. Volk. 1993. Biological responses of a tundra river to fertilization. Ecology 74: 653-672.

Peterson, B.J., W.M. Wollheim, P.J. Mulholland, J.R. Webster, J.L. Meyer, J.L. Tank, E. Marti, W.B. Bowden, H.M. Valett, A.E. Hershey, W.H. McDowell, W.K. Dodds, S.K. Hamilton, S.V. Gregory and D.D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292: 86-90.

Rosemond, A.D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia 94: 585-594.

Rosemond, A.D., P.J. Mulholland and S.H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Canadian Journal of Fisheries and Aquatic Sciences 57: 66-75.

Rosemond, A.D., P.J. Mulholland and J.W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74: 1264-1280.

Rosen, B.H. and R.L. Lowe. 1984. Physiological and ultrastructural responses of *Cyclotella meneghiniana* (Bacillariophyta) to light intensity and nutrient limitation. Journal of Phycology 20: 173-183.

Scott, M.C., G.S. Helfman, M.E. McTammany, E.F. Benfield and P. Bolstad, V. 2003. Multiscale influences on physical and chemical stream conditions across Blue Ridge landscapes. Journal of the American Water Resources Association 38: 1379-1392.

Selby, M.J. 1985. Earth's Changing Surface: An Introduction to Geomorphology. Clarendon Press, Oxford.

Shortreed, K.S., A.C. Costella and J.G. Stockner. 1984. Periphyton biomass and species composition in 21 British Columbia lakes: seasonal abundance and response to whole-lake nutrient additions. Canadian Journal of Botany 62: 1022-1031.

Steinman, A.D. 1992. Does an increase in irradiance influence periphyton in a heavily-grazed woodland stream? Oecologia 91: 163-170.

Steinman, A.D., C.D. McIntire, S.V. Gregory, and G.A. Lamberti. 1989. Effects of irradiance and grazing on lotic algal assemblages. Journal of Phycology 25: 478-485.

Stevenson, R.J., R. Singer, D.A. Roberts and C.W. Boylen. 1985. Patterns of epipelic algal abundance with depth, trophic status, and acidity in poorly buffered New Hampshire Lakes. Canadian Journal of Fisheries and Aquatic Sciences 42: 1501-1512.

Swank, W.T. and D.A. Crossley. 1988. Forest Hydrology and Ecology at Coweeta. Springer-Verlag, New York.

Tank, J.L. and W.K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48: 1031-1049.

Tilman, D. 1977. Resource competition between planktonic algae: an experimental and theoretical approach. Ecology 58: 338-348.

Triska, F.J., V.C. Kennedy and R.J. Avanzino. 1983. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. *In* Dynamics of Lotic Ecosystems. *Edited by* Fontaine, T.D.I. and S.M. Bartell. Ann Arbor Science Publishers, Ann Arbor, Mich. pp. 129-159.

Wellnitz, T.A., R.B. Rader and J.V. Ward. 1996. Importance of light and nutrients in structuring an algal community in a rocky mountain stream. Journal of Freshwater Ecology 11: 399-413.

Winterbourn, M.J. 1990. Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. Freshwater Biology 23: 463-474.

Table 3.1. Physical and chemical characteristics of the reference stream and enriched stream from CHL. Discharge, and temperature are from July 1999-July 2002. Nutrient and pH data are from the pretreatment period July 1999- July 2000.

		Reference	Treatment
Catchment	Area (ha)	5.2	5.5
	Elevation (m a.s.l.)	820	841
Channel	Gradient (cm m^{-1})	27	33
	Length (m)	145	282
	Bankfull area (m^2)	327	443
Temperature (°C)	Daily mean (*n*)	12.0 (336)	12.0 (336)
	Range	2.6-18.6	4.8-16.7
Discharge (L s^{-1})	Daily mean (*n*)	0.32 (1114)	0.53 (1114)
	Range	0.006-3.8	0.06-4.8
pH	Mean (*n*)	6.59 (24)	6.87 (18)
	Range	6.2-7.0	6.6-7.9
NO_3-N (μg L^{-1})	Mean (*n*)	15.4 (5)	18.8 (12)
	Range	9.4-25.8	4.0-39.5
NH_4-N (μg L^{-1})	Mean (*n*)	9.4 (4)	9.9 (12)
	Range	0-30.4	0-24.9
SRP (μg L^{-1})	Mean (*n*)	7.6 (5)	8.8 (12)
	Range	0-20.3	0-22.1

Table 3.2. Average pretreatment (July 1999 – July 2000) and treatment (July 2000 – July 2002) nutrient levels (μg/L) for the reference and treatment streams. SRP=soluble reactive phosphorus; BD = below detection.

Site	$(NO_2^-+NO_3^-)$-N	NH_4^+-N	SRP	Molar DIN:SRP
Pre enrichment				
Reference				
Mean (1 SD)	**15.4** (6.6)	**9.4** (14.1)	**7.6** (8.0)	7.2
Range (*n*)	9-26 (5)	BD-30 (5)	BD-20 (5)	
Treatment				
Mean (1 SD)	**18.8** (11.5)	**9.9** (8.6)	**8.8** (8.1)	7.2
Range (*n*)	4-40 (12)	BD-25 (12)	BD-22 (12)	
Post enrichment				
Reference				
Mean (1 SD)	**16.9** (29.8)	**10.4** (16.9)	**3.7** (4.7)	16.3
Range (*n*)	BD-151 (33)	BD-76 (33)	BD-17 (33)	
Treatment				
Mean (1 SD)	**308.9** (377.8)	**105.5** (119.7)	**51.2** (55.6)	17.9
Range (*n*)	11-1711 (44)	6-566 (44)	BD-268 (44)	

Table 3.3. Average cell biovolume ($\mu m^3\ cm^{-2}$) and standard deviations of all cells and algal divisions for reference and treatment streams during the pretreatment year and both years of nutrient enrichment.

Site	**Total**	Diatoms	Chrysophyta	Chlorophyta	Cyanobacteria
Pre enrichment					
Reference					
Mean	**1.35×10^6**	1.3×10^6	21,259	1,004	116
SD (*n*=30)	(0.72×10^6)	(0.72×10^6)	(44,721)	(2,460)	(285)
Treatment					
Mean	**1.90×10^6**	1.89×10^6	13,632	0	0
SD (*n*=30)	(1.44×10^6)	(1.45×10^6)	(21,201)	(1)	(1)
Post enrichment Year 1					
Reference					
Mean	**1.49×10^6**	1.45×10^6	28,547	423	252
SD (*n*=29)	(1.10×10^6)	(1.12×10^6)	(39,317)	(1,037)	(617)
Treatment					
Mean	**2.71×10^6**	2.70×10^6	427	4,721	15
SD (*n*=29)	(1.00×10^6)	(1.00×10^6)	(744)	(11,565)	(37)
Post enrichment Year 2					
Reference					
Mean	**3.22×10^6**	3.18×10^6	33,631	1,865	550
SD (*n*=29)	(4.42×10^6)	(4.43×10^6)	(46,535)	(2,515)	(953)
Treatment					
Mean	**1.65×10^6**	1.65×10^6	403	4,714	23
SD (*n*=30)	(1.23×10^6)	(1.23×10^6)	(987)	(11,546)	(57)

Table 3.4. Algal taxa encountered from periphyton samples on tiles. Categories based on average relative biovolume across all samples: A = abundant (>20%), C = common (5-20%), U = uncommon (1-5%), R = rare (<1%) of relative biovolume. No letter means that the species was not detected in that stream. Combined species in bold accounted for 95% of the biovolume and were analyzed for responses to nutrient enrichment (Fig. 3.4).

	Reference	Treatment
BACILLARIOPHYTA		
Achnanthes deflexa Reim.		R
Achnanthes stewartii Patr.	R	R
Achnanthes subrostrata var. *appalachiana* Camburn & Lowe	U	U
Achnanthidium minutissimum (Kütz.) Czarn.	R	R
Cymbella tumida (Breb ex Kütz.)	R	
Diatoma hiemale var. *mesodon* (Ehr.) Grun.	R	R
Encyonema minutum (Hilse) Mann	R	R
Eunotia curvata (Kütz.) Lagerst. var. *curvata*	R	R
Eunotia exigua (Breb. ex Kütz.) Rabh. var. *exigua*	R	R
***Eunotia pectinalis* var. *minor* (Kütz.) Rabh.**	**A**	**A**
***Eunotia pectinalis* var. *recta* A. Mayer ex Patr.**	**C**	**C**
Fragilaria vaucheriae (Kütz.) Peters.	R	R
Frustulia rhomboidies (Ehr.) De T.	R	R
Gomphonema acuminatum var. *pusillum* Grun.	R	R
Gomphonema gracile Ehr.	R	R
***Gomphonema parvulum* (Kütz.)**	**C**	**C**
Melosira varians Ag. var. *varians*		R
***Meridion constrictum* Ralfs**	**A**	**A**
Navicula angusta Grun.	R	R
Navicula placenta Ehr.	U	R
***Navicula tantula* Hust.**	**U**	**C**
Nitzschia palea (Kütz.) W. Sm.	R	R
Nizschia dissipata (Kütz.) Grun.	R	R
Pinnularia mesogongyla Ehr.	R	R
Pinnularia subcapitata var. *paucistriata* (Grun.) Cl.	R	
Planothidium lanceolatum (Breb.) Round & Buk.	R	R
Surirella angusta Kütz.	R	R
Synedra minuscula Grun.	R	R
Synedra rumpens var. *meneghiniana* Grun.	R	R
Synedra ulna (Nitz.) Ehr.	R	R
CHRYSOPHYTA		
Unidentified stomatocysts	U	R

Table 3.4, cont'd

	Reference	Treatment
CHLOROPHYTA		
Mougeotia sp.	R	R
CYANOBACTERIA		
Chamaesiphon sp.	R	R
Unidentified spheres	R	R
Unidentified filaments	R	R

Figure 3.1. Algal biomass ±1SE as chlorophyll *a* (A) and AFDM (B) from the reference (Ref) and treatment (Tmt) stream. P-values are from RIA on differences between streams before and after enrichment.

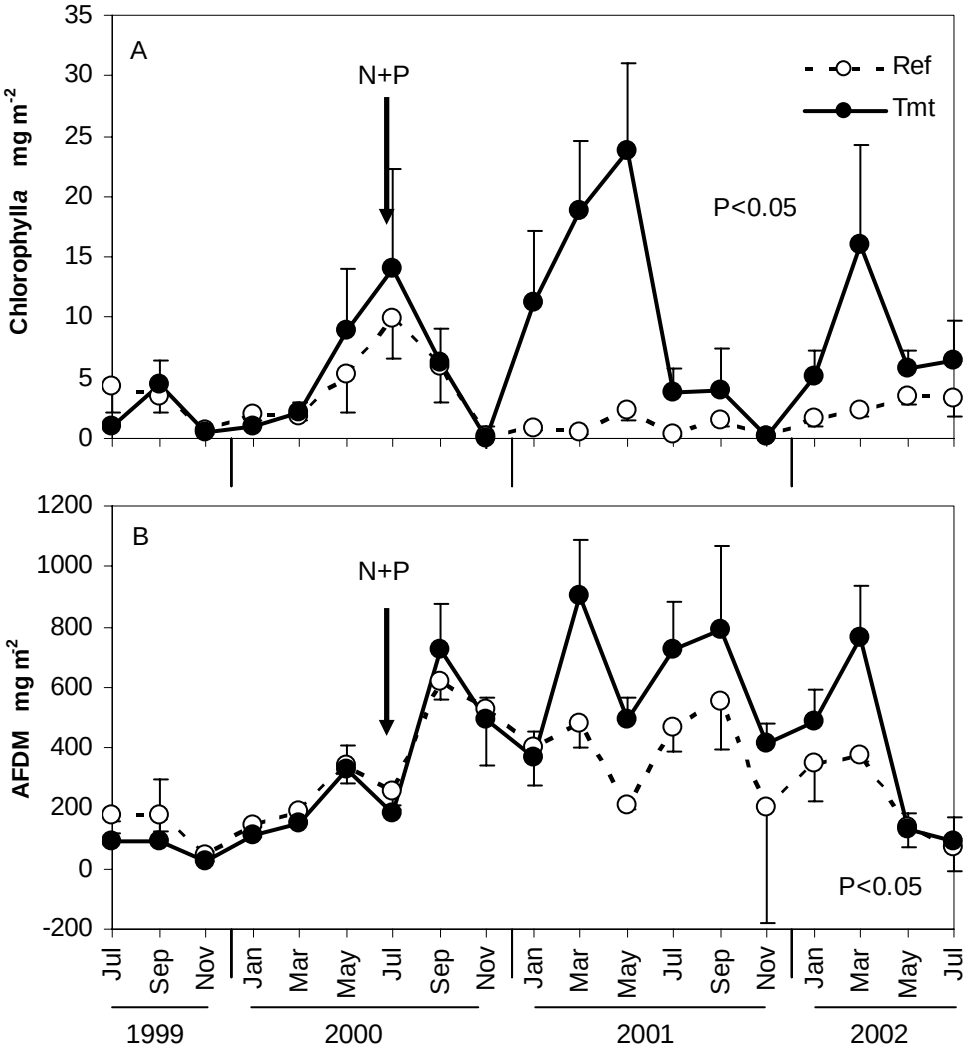

A
N+P
Ref
Tmt
P<0.05
Chlorophylla mg m-2
35
30
25
20
15
10
5
0
B
N+P
P<0.05
AFDM mg m-2
1200
1000
800
600
400
200
0
-200
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
1999
2000
2001
2002

Figure 3.2. Algal biomass ±1SE as total biovolume (A) and chlorophyll *a* per biovolume (B) from the reference (Ref) and treatment (Tmt) stream. RIA showed that differences between streams before and after enrichment were not significantly (n.s.) different.

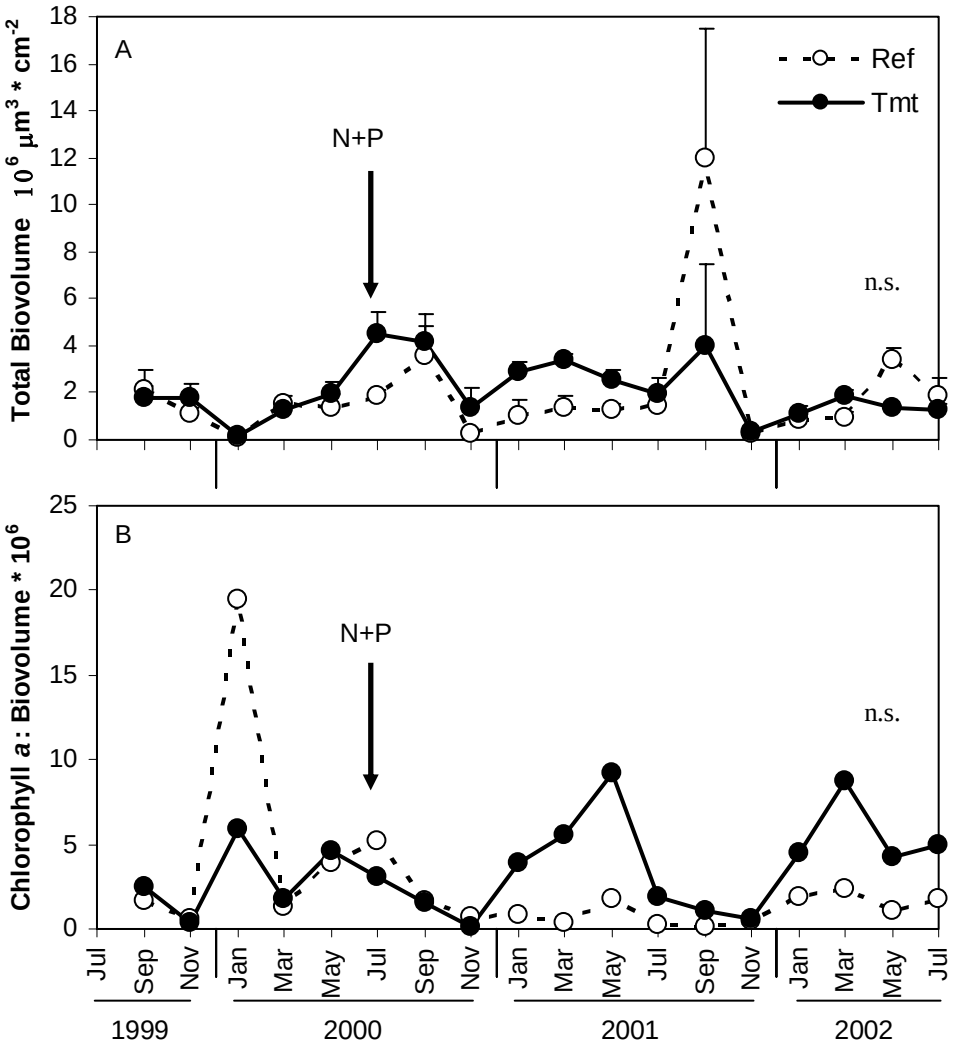

A
Ref
Tmt
N+P
n.s.
Total Biovolume 10^6 µm^3 * cm^-2
B
N+P
n.s.
Chlorophyll a: Biovolume * 10^6
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
1999
2000
2001
2002

Figure 3.3. Average chlorophyll *a* (A), PAR (B), AFDM (C) and total biovolume (D) ±1SE in the reference (Ref) and treatment (Tmt) streams during seasonal periods of high and low levels of chlorophyll *a* in the treatment stream. Different letters above the bars indicate Kruskall-Wallis $p<0.05$ and Student's *t* multiple comparison test $p<0.05$.

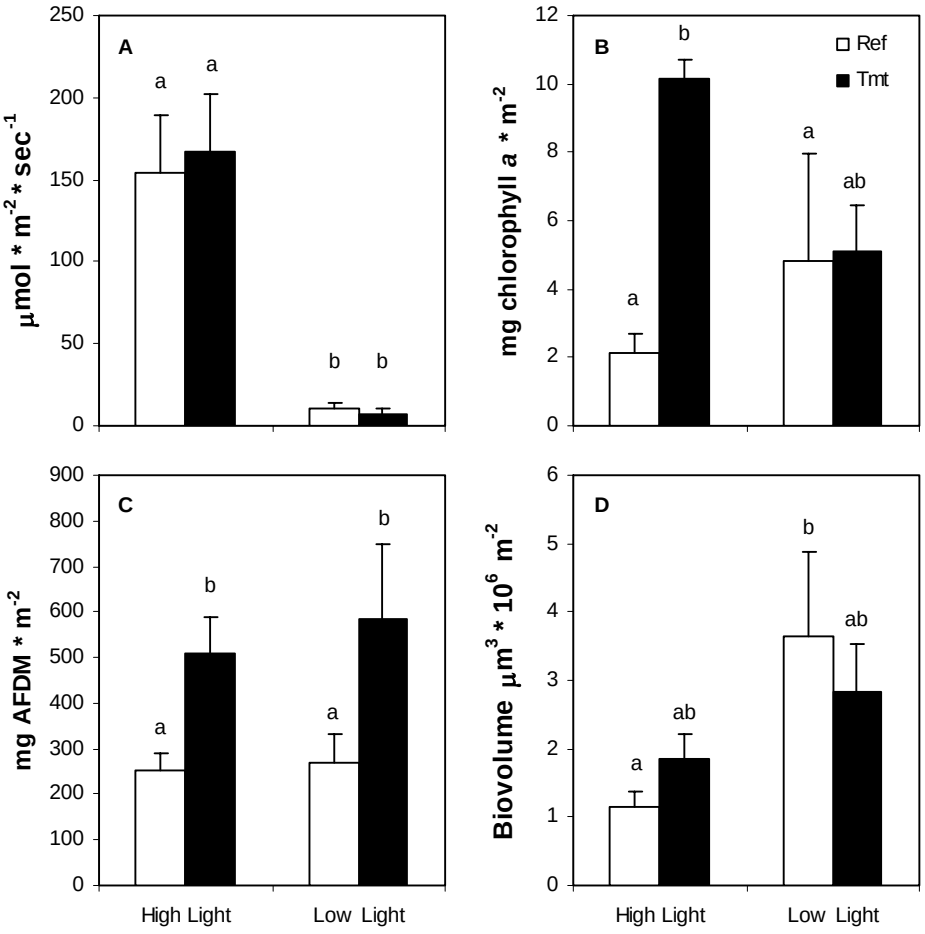

A
B
C
D
Ref
Tmt
µmol * m-2 * sec-1
mg chlorophyll a * m-2
mg AFDM * m-2
Biovolume µm3 * 10^6 m-2
High Light
Low Light
a
b
ab

Figure 3.4. Increase in cell density per day ±1SE between week 2 and week 4 of the algal growth experiment during March and April (A) and June and July (B) trials. Significant differences between streams determined with a *t*-test (*$p < 0.05$).

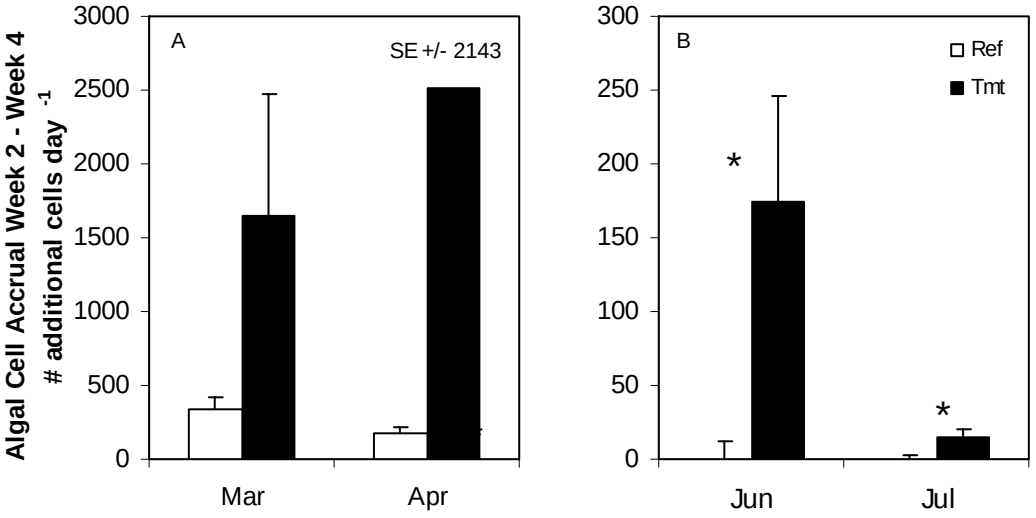
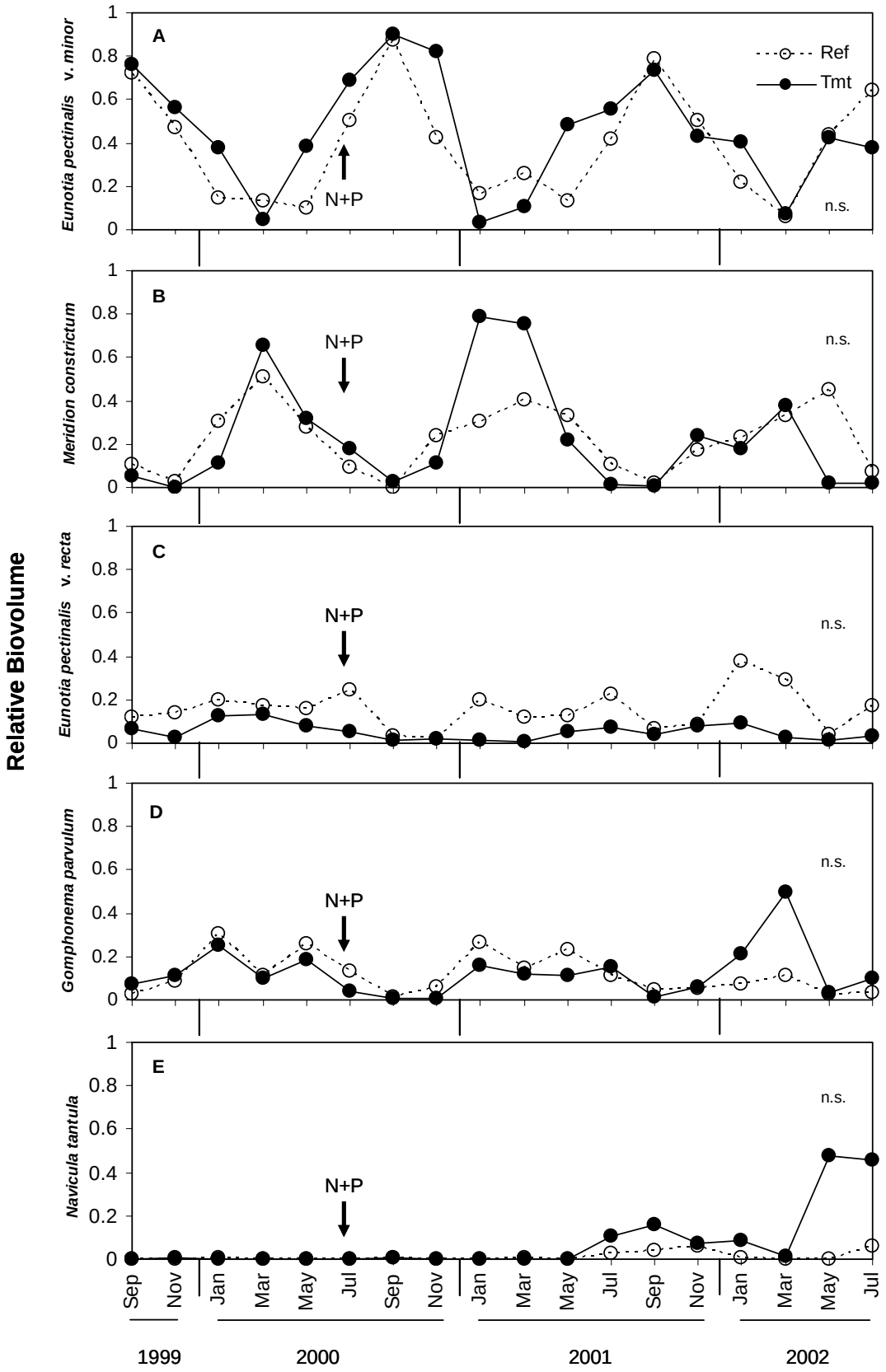

Algal Cell Accrual Week 2 - Week 4
additional cells day -1
3000
2500
2000
1500
1000
500
0
A
SE +/- 2143
Mar
Apr
300
250
200
150
100
50
0
B
Ref
Tmt
*
*
Jun
Jul

Figure 3.5. Relative biovolume of the 5 most common species: *Eunotia pectinalis* var. *minor* (A), *Meridion constrictum* (B), *Eunotia pectinalis* var. *recta* (C), *Gomphonema parvulum* (D), and *Navicula tantula* (E) from the reference (Ref) and treatment (Tmt) stream. RIA showed that differences between streams before and after enrichment were not significantly (n.s.) different.


Relative Biovolume
A
Eunotia pectinalis v. minor
Ref
Tmt
N+P
n.s.
B
Meridion constrictum
N+P
n.s.
C
Eunotia pectinalis v. recta
N+P
n.s.
D
Gomphonema parvulum
N+P
n.s.
E
Navicula tantula
N+P
n.s.
0
0.2
0.4
0.6
0.8
1
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
1999
2000
2001
2002

CHAPTER 4

VARIATION IN PERIPHYTIC ALGAL ASSEMBLAGES ON BRYOPHYTE AND BEDROCK SUBSTRATA: POTENTIAL FOR DIFFERENTIAL RESPONSE TO ENVIRONMENTAL VARIATION [1]

[1] Greenwood, J.L., A.D. Rosemond and S.N. Bland. To be submitted to *Journal of the North American Benthological Society*.

Abstract

Algae grow on a variety of substrata and their response to environmental variables may be substratum-dependent. In order to examine the potential effects of variation in nutrient and light availability on periphyton from different substrata, algal biomass (as biovolume) and algal assemblage composition were examined on moss, liverwort and bedrock substrata. The comparison among substrata was conducted in two forested headwater streams, one nutrient enriched and one control, sampled from months with high (May) and low (July) light availability. Bryophytes (both the moss and liverwort) supported 2 x 10^6 greater periphytic algal biovolume than bedrock. Bryophyte biomass was not affected by nutrient enrichment; therefore, the potential for bryophytes as a substratum for epiphytic colonization did not increase. Algal assemblage composition was different among the three substrata with diatoms dominating on all substrata, and filamentous cyanobacteria and *Audouinella* sp. (Rhodophyta) showing a stronger presence on bedrock. Periphyton biovolume was generally higher in May than July on all substrata. Ordination analysis and tests of individual species response indicated that response by individual taxa, when they occurred, were positive in regard to light availability (inferred from month sampled), were negative in regard to inferred nutrient availability, and were inconsistent for a given taxon across substratum types. Periphyton assemblages associated with liverworts differed less than those associated with bedrock and moss due to environmental variation. In these headwater streams, periphyton communities differed in biomass and composition across substratum types. Response to environmental variation was driven primarily by individual periphyton species response, although the responses were not consistent across substratum types. Thus, variability in substratum types in headwater streams appears to support greater diversity of

periphyton and response to environmental change than would be observed from a single substratum.

Introduction

The nature of periphytic algal assemblages in streams can vary depending on the substratum to which they are attached (Burkholder 1996). Bryophytes can be an important component of the benthos in many streams (Naiman 1983; Slack and Glime 1985; Glime and Vitt 1987; Steinman and Boston 1993; Suren 1993; Cattaneo and Fortin 2000), and hence, an important substratum for periphyton (Douglas 1958; Pentecost 1991; Rothfritz et al. 1997; Group 1999; Passy et al. 1999; Slavik et al. 2004). In general, when stream periphyton response to environmental variation in streams is studied, periphyton growing epiphytically on bryophytes is rarely assessed. More frequently, epilithic periphyton (e.g. Welch et al. 1988; Duncan and Blinn 1989; Lohman et al. 1991; Rosemond 1994; Slavik et al. 2004; references in Borchardt 1996) or periphyton colonized on tiles (e.g. Mulholland and Rosemond 1992; Rosemond et al. 1993) are the habitats of choice to detect experimental effects.

Rock substrata and bryophytes can be qualitatively different substrata for periphyton. This is due to various factors such as gross physical structure, surface micro-ultrastructure, or the fact that macrophytes can leak nutrients or allelopathic chemicals from their surfaces (Burkholder 1996). Differences in biomass and species distributions of algal epiphytes on bryophytes vs. rock substrata have not been frequently examined, but some differences between epiphytes and epilithon have emerged. For instance, bryophytes can potentially sustain substantially higher algal biomass levels compared to bare rock (Arscott et al. 1998; Chantha et al. 2000; Slavik et al. 2004). Also, in some of the few studies that have examined algal assemblage composition on bryophytes, bryophytes have been shown to support significantly different algal communities

compared to rock substrata in the same ecosystem (Pentecost 1991; Rothfritz et al. 1997; Passy et al. 1999; Lim et al. 2001). Further, algal assemblages can also differ between moss and liverwort species (Pentecost 1998) or among different moss species (Pentecost 1991; Stream Bryophyte Group 1999) in the same ecosystem. Thus, interpreting the response of the entire periphyton community to experimental manipulation based on measures of strictly epilithic periphyton may miss important yet altogether different contributions of epiphyton.

Nutrient and light availability are important factors that contribute to the development of algal biomass and assemblage composition (Stevenson et al. 1996). Generally, studies (mostly on epilithic periphyton) have shown that nutrients and light can have positive effects on periphyton biomass and can alter species assemblages of epilithic communities. Studies comparing nutrient effects between substrata are rare but have shown relatively greater nutrient effects on periphyton from inorganic substrata such as rock vs. organic substrata such as wood or sediment (Vadeboncoeur and Lodge 2000; Tank and Dodds 2003). The reasoning behind this is that periphyton growing on organic substrata can often obtain nutrients directly from their substratum, depending less on water column nutrients, and therefore showing less of a response to nutrient enrichment compared to epilithic periphyton which would rely much more on external nutrients. Few studies have examined or shown strong differences in periphyton community response to light availability across different substrata (Albay and Akcaalan 2003). Potentially, shading within the complex structure of bryophytes may lead to stronger light limitation for epiphytes relative to epilithic periphyton. Thus, epiphytic periphyton could respond more strongly to increases in light relative to epilithic communities whose substratum has a simpler physical structure. Also, we know very little about the potential interactive effects that nutrients and light may have across different substrata. Nutrients and light have been simultaneously

manipulated to examine the effects on epilithic periphyton (Hill and Knight 1988; Rosemond 1993), but rarely with multiple substrata (Vadeboncoeur and Lodge 2000). Examining how periphyton from bryophyte and bedrock substrata in the same system respond to variation in nutrient and light availability potentially yields insights into substratum-dependent response of periphyton to environmental manipulation.

An important consideration in the comparison of how nutrient enrichment affects epiphytes vs. epilithon is that the amount of bryophyte available as a substratum can potentially increase in response to nutrient enrichment. For instance, bryophyte biomass increased 18-fold after seven years of phosphorus enrichment in the Kuparuk River, AK (Bowden et al. 1994). In fact, the moss response to enrichment ultimately resulted in profound effects on the structure of the entire stream food web (Slavik et al. 2004). Thus, if bryophytes themselves respond to nutrient enrichment, the potential area available for periphyton colonization will also increase, and the response of periphyton as epiphytes may outweigh the response of epilithic periphyton.

Few studies have examined the response of epiphytic algal biomass and community structure on bryophytes to experimental nutrient enrichment (Stream Bryophyte Group 1999). However, from studies of angiosperm macrophytes, it is clear that nutrient enrichment can result in increased epiphytic algal biomass (Lalonde and Downing 1991; Neckles et al. 1993; Neundorfer and Kemp 1993) and shifts in community structure (Coleman and Burkholder 1994; Coleman and Burkholder 1995). In the Kuparuk River study, phosphorus enrichment resulted in significant increases in chlorophyll *a* from bryophyte epiphytes which attained levels almost double those measured for algal epilithon (Slavik et al. 2004). However, in a phosphorus enrichment in eastern Tennessee, no changes were found in epiphyte abundance or community structure, potentially due to heavy grazing pressure (Steinman 1994). Further examination of the

response of algal epiphytes on aquatic bryophytes to nutrient enrichment is clearly needed as patterns of algal community response in bryophytes compared to other substrata are not currently well understood.

Bryophytes are frequently found in heavily shaded streams where periphyton biomass is low (Steinman and Boston 1993). Thus, light limitation can play an important role in the development of algal epiphyte communities. There is some evidence that light can play a role in the development of epiphytic biomass. In studies in New Zealand, chlorophyll *a* measured from artificial bryophytes from a shaded stream supported a fraction of the chlorophyll levels compared to an unshaded stream (Suren 1992a; Suren 1992b) and light availability also played a role in structuring the epiphytic diatom communities (Suren, 1992a). Thus, examination of the role of seasonal light availability is an important aspect of the potential for bryophyte epiphytes to respond to nutrient enrichment. Seasonal light availability has been shown to play a role in how epilithic algae respond to nutrient enrichment in shaded streams (Hill and Knight 1988; Rosemond et al. 2000; Chapter 3).

This study was conducted to determine whether periphyton assemblages differed significantly on bryophyte and bedrock substrata in headwater streams. We also tested whether bryophyte substrata increased in biomass in response to long-term nutrient enrichment. The long-term enrichment additionally allowed us to assess whether periphyton assemblages on different substrata responded differently to enrichment, which was assessed during periods of seasonally high and low light. We predicted that bryophytes would support more periphyton biomass vs. bedrock per area of streambed and periphyton assemblages would differ across substrata. We also predicted that bryophyte biomass would increase with nutrient enrichment, thus increasing the amount of substrata available for epiphyte colonization. However, regardless of substrata, we

predicted that biomass of periphyton would be highest with high nutrient and light availability. Further, we predicted the effects of nutrient availability would be stronger for inorganic bedrock and effects of light availability may be stronger for epiphytic periphyton, which grow on a more physically complex structure.

Methods

Study Site

An experimentally enriched headwater stream, Catchment 54 (C 54) and two reference headwater streams, Catchment 53 (C 53) and Catchment 56 (C 56), were examined at the Coweeta Hydrologic Laboratory (CHL), a USDA Forest Service research facility located in the Blue Ridge Mountain physiographic province in the southern Appalachian mountains (North Carolina, U.S.A.). The same treatment stream (C 54) was used throughout the study, but separate streams were used for a bryophyte biomass control (C 56) and for control comparisons of epiphytic assemblage composition (C 53). Two reference streams were used in order to reduce researcher impact on C 53, which was used as a reference stream for two research projects. All three streams have similar physical and chemical characteristics (Table 4.1). Dominant vegetation includes tulip poplar (*Liriodendron tulipifera* L.), white oak (*Quercus alba*, L.), red oak (*Quercus rubra*, L.), red maple (*Acer rubrum*, L.), and dogwood (*Cornus florida*, L.) (Swank and Crossley 1988). Rhododendron (*Rhododendron maximum* L.), an evergreen, grows as a dense understory in the riparian zone. Thus, a "double canopy" of deciduous trees and rhododendron shades the study streams to some degree during all seasons. Seasonal differences occur in light availability in the streams used to assess periphyton communities. Average

instantaneous light levels were higher in May (C 53 ~200 μmol m^{-2} s^{-1} and C 54 ~100 μmol m^{-2} s^{-1}) compared to July (C 53 and C54 ~10μmol m^{-2} s^{-1}) (Chapter 3).

Nutrient Enrichment

Pretreatment data were collected from the treatment stream from July 1999-July 2000, and beginning 11 July 2000, was continuously enriched with NH_4NO_3, KH_2PO_4 and K_2HPO_4 along the entire 150m length of the study reach for two years. A dissolved nutrient salt solution was pumped into an irrigation line (approx. 2cm diameter) that was fed with stream water from an upstream head tank. The line ran the entire length of study reach adjacent to the streambed while multiple spigots inserted along the length of the hose delivered the nutrient solution into the stream. A metering pump (Liquid Metronics, Inc.) was electronically linked with a Campbell data logger to an ISCO flow measurement device at the downstream end of the stream reach to deliver nutrients in a discharge-dependent manner. Stream water nutrient concentrations were measured during the pretreatment and experimental periods. During the pretreatment period, one to four samples were taken from the reference and treatment streams on five sampling dates in the reference stream and 12 sampling dates in the treatment stream. During the enrichment period, one sample was taken from the reference stream and five samples were taken along the length of the treatment stream approximately every two weeks to confirm that nutrients were elevated in an even distribution within the study reach. Stream water samples were filtered with Millipore HA filters into acid-washed bottles and frozen until analysis. Concentrations of NO_2^--N, NO_3^--N, NH_4^+-N and soluble reactive phosphorus (SRP) were determined with an Alpkem Rapid Flow Analyzer 300 at the University of Georgia Chemical Analysis Laboratory.

Assessment of Algal Epiphytes

Two dominant bryophyte species were common enough to obtain multiple samples for epiphyte community analysis from both the treatment C 54 and reference stream C 53, the moss *Platylomella lescurii* (Sull.) Andrews, and the liverwort *Jubula pennsylvanica* (Steph.) Evans. Three bryophyte samples were taken May 1, 2002 and July 10, 2002, seasons of high and low light availability, respectively. An area of $1cm^2$ was scraped from bedrock with a knife and the entire sample was fixed in 5% formalin in a 20mL scintillation vial. Algal species composition of epiphytes was determined by assessing the loosely and tightly adhered species separately. Loose epiphytes were assessed by shaking the vial vigorously 100 times, and the species composition was determined from the resulting slurry by counting at least 500 cells in a Palmer-Maloney nannoplankton counting chamber at 400X. The community of tightly adhering epiphytes was determined by removing individual leaflets from the plant, placing them in a Palmer-Maloney counting chamber, and counting all algal cells on at least 10 leaflets at 400X. Diatom species were identified after cleaning with bleach (Carr et al. 1986) to clear cell contents and permanent mounting in Naphrax high resolution mounting medium. To calculate algal cell biovolume, average dimensions of up to 10 cells in each taxon were applied to standard geometric shapes that best represented the shape of each taxon (Hillebrand et al. 1999). Biovolumes from the Philadelphia Academy database http://diatom.acnatsci.org/autecology/#browse were used to estimate biovolumes for rarely encountered taxa. Algal biovolume on bryophytes were initially calculated as number of cells per length of bryophyte and then standardized per cm^2 of bedrock. Although this method of calculation does not allow a direct comparison of how much periphyton is supported per surface area of substratum, it allows for comparison of how much algal growth can be supported per unit of streambed with and without bryophyte growth.

Bedrock Sampling

Periphyton was sampled from 3 areas of bedrock in the treatment stream and reference stream C 53 on 14 May and 15 July 2002. A ~6cm length of ~5cm diameter PVC tube was skirted on one end with neoprene so that a seal could be formed between the PVC tube and the bedrock surface. Once a seal was formed, a test tube brush was used to detach periphyton with ~50 mL of stream water creating a slurry. The entire slurry was then removed with a turkey baster. A portion of the sample was fixed in 5% formalin for determination of algal assemblage composition which was performed by counting at least 500 cells in a Palmer-Maloney nannoplankton counting chamber at 400X. Diatom species were identified and biovolumes were calculated as with bryophyte epiphytes.

Bryophyte Biomass

Estimates of moss biomass were measured monthly October 1999 – August 2002 (concurrent with the one year of pre-treatment and 2 years of enrichment in the treatment stream) except in Jan. and June 2001 due to logistical problems. Percent cover of moss was estimated from six 45cm x 50cm permanent quadrats on exposed bedrock in both the treatment stream C 54 and in the reference stream C 56. Biomass was estimated by laying a sheet of plexiglass divided in 90 (arranged in a 9x10 array) 5x5 cm squares on each permanent quadrat and scoring (values of 1, 3 5, 7, 9 or 10) based on estimated percent cover, where 1=10%, 3=30%, 5=50%, 7=70%, 9=90% and 10=100% bryophyte cover. Eight times throughout the experimental period, for each percent cover estimation, a haphazard location on bedrock (but off the permanent quadrats) of approximately 20 cm^2 was sampled to relate per cent cover to biomass. All bryophyte material was removed with forceps, and AFDM determined after combustion at 500°C. A regression of

biomass and per cent cover was determined to estimate bryophyte biomass from the quadrat percent cover estimations.

Data analysis

Overall differences in species composition among samples was assessed using the ordination technique non-metric multidimensional scaling (NMS) using the Bray-Curtis distance measure with the statistical software PC-ORD 4 (MjM Software Design, Gleneden Beach, OR, U.S.A.). NMS is considered a more robust method for dealing with ecological data compared to other commonly used ordination techniques such as principal components analysis (Minchin 1987; McCune and Grace 2002). Arcsine square root transformed relative biovolume species data were used in the NMS analysis. Rare taxa present in less than 2 samples were excluded from analysis. Randomized intervention analysis (Carpenter et al. 1989), or RIA, was used to assess differences in bryophyte biomass between the reference and treatments streams. RIA detects changes of variables in a time series between unreplicated reference and treatment systems. Effects of substratum, stream (enriched vs. reference) and season (high light vs. low light) were assessed for total biovolume was using a 3-factor analysis of variance (ANOVA). Multiple comparisons among substrata were conducted with a Tukey test. Biovolume data were normalized by log (X+1) transformation. In order to examine overall effects of substratum, stream and season on important taxa, log-transformed biovolume for taxa comprising at least 10% of the biovolume on at least one substratum was also assessed with 3-factor ANOVA. In order to examine effects of stream and season on communities within each substratum, log-transformed biovolume of dominant taxa were analyzed with 2-way ANOVAs by examining each substratum independently with stream and season as the effects within treatment. Taxa that did not compose

at least 10% of the biovolume for a particular substratum were not analyzed for that substratum. Cyanobacterial filaments were grouped for both the 3-way and 2-way ANOVAs.

Results

Nutrient Enrichment

The enrichment increased DIN ~13X and SRP ~5X to approximately 400µg/L and 50 µg/L SRP in the treatment stream (Table 4.2), resulting in a stream water molar N:P ratio of about 17.9:1. Stock solution was added at a molar N:P ratio of approximately 11.4:1, which suggests that there was a relatively greater uptake of P. These nutrient concentrations are within range of those found regionally in streams (Scott et al. 2003). Nutrient levels in the reference stream remained consistent across pretreatment and treatment periods with DIN <30µg/L and SRP <10µg/L.

Multivariate Assessment of Algal Epiphyte Assemblages

Ordination with NMS resulted in 3 axes explaining a total of 86.6 % of the variance in species composition among the samples. The second and third axes explained the most variance (43.2% and 23.6% respectively) and were used to generate a scatterplot of samples in species space (Figs. 4.1, 4.5-4.6). In this type of plot, sample points that are close together have more similar species assemblages than sample points that are farther apart. Our plot shows that samples from the same substratum tended to cluster together (Fig. 4.1), indicating that each substratum supported a relatively unique algal assemblage structure.

Taxa that correlated with any of the three NMS axes with at least r^2=0.30 were examined with bubble plots (Fig. 4.2). Each point on the bubble plot is a sample point in species space which corresponds to the same sample point on the NMS scatterplot (Fig. 4.1). The size of the

bubble represents the relative biovolume of that species in that sample (i.e. larger bubbles mean higher relative biovolume than smaller bubbles). Six taxa had at least $r^2 = 0.30$, and these taxa tended to show preference for a particular type of substratum, confirming the trends in separation based on substratum type in the NMS plot (Fig. 4.1). *Achnanthes subrostrata* var. *appalachiana* (Fig. 4.2A) was most common on liverwort substrata, while *Eunotia pectinalis* v. *recta* (Fig. 4.2B) and *Gomphonema parvulum* (Fig. 4.2D) were most common on moss and liverwort. *Frustulia rhomboides* (Fig. 4.2C) and *Audouinella* sp. (Fig 4.2F) were more common on bedrock. *Meridion constrictum* (Fig. 4.2E) was the only taxon highly correlated with the unplotted first NMS axis and had high relative abundances on all 3 substrata compared to the other taxa. Also, bryophytes supported more algal taxa (27 and 28 taxa for the moss and liverwort, respectively) compared to bedrock (16 taxa; Table 4.5).

Bryophyte Biomass

The relationship between biomass scores and AFDM was described by an exponential line fit ($y = 1.4981x^{1.619}$, $R^2 = 0.84$) which was used to estimate biomass from plot scores. Although biomass was on average higher in the reference stream, there were no significant differences in moss biomass with enrichment between the treatment and the reference streams (Fig. 4.3; RIA $p>0.05$). Seasonal patterns in bryophyte biomass variation were not apparent.

Overall effects of nutrient and light availability on algal biovolume

Total algal biovolume per area of streambed across the 3 substrata (Fig. 4.4) was significantly lower on bedrock than on either moss or liverwort (ANOVA $p \leq 0.0001$; Table 4.3), and total biovolume for both moss and liverwort was, on average, 2.5×10^6 times higher than on bedrock. Total biovolume did not significantly differ with stream, but was significantly higher during the season of high light availability ($p \leq 0.05$; Table 4.3).

Taxonomic response to nutrient and light availability

The NMS plot was used to gauge general trends in stream and season effects on algal assemblages from the 3 different substrata by outlining data points from samples within the same stream (Fig. 4.5) and from the same sampling date (Fig. 4.6). Regarding differences between streams, all samples in the reference stream tended to cluster together (Fig. 4.5). The bryophytes from the enriched stream overlapped with a few samples from the reference stream but were well separated from the bedrock samples from the enriched stream, and differences among substrata seemed to be stronger in the enriched stream (Fig. 4.5). Regarding effects of season, samples from all substrata did not show a strong separation based on sampling month. Bedrock samples from both sampling dates separated strongly from each other and from the bryophyte samples. Conversely, moss and liverwort samples showed overlap within each substratum between the low light season and the high light season samples. Overall, bedrock showed the strongest within-substratum separation of algal species assemblages due to both differences between streams and between months.

Overall, diatoms dominated assemblages on all substrata, and cyanobacteria and the red alga *Audouinella* sp. were common only on bedrock (Fig. 4.7; Tables 4.4, 4.5). Dominant taxa on all substrata include: *A. subrostrata* var. *appalachiana*, *Eunotia maior*, *Eunotia pectinalis* v. *minor*, *F. rhomboides* and *M. constrictum*. Taxa that were additionally dominant on the moss were *Gomphonema acuminatum* v. *pusillum* and *G. parvulum*, while *G. parvulum* was also dominant on bedrock.

Significant differences for absolute biovolume of the dominant species in terms of substratum almost always showed lowest biovolume on bedrock, which occurred for *A. subrostrata* var. *appalachiana*, *Eunotia pectinalis* v. *minor*, *G. acuminatum* v. *pusillum* and *G.*

parvulum (Table 4.6). Absolute biovolume for *G. acuminatum* v. *pusillum*, *G. parvulum*, and cyanobacteria filaments was also higher for those species on moss compared to liverwort (Table 4.6). Biovolume was significantly lower in the enriched stream relative to the reference stream for *Achnanthidium minutissimum*, *F. rhomboides*, *G. acuminatum* v. *pusillum*, *G. parvulum*, and *M. constrictum* (Table 4.6). Biovolume was not significantly higher for any taxa in the enriched stream. Biovolume was significantly higher during the season of high light vs. low light availability for *A. minutissimum*, *F. rhomboides, G. parvulum* and *M. constrictum*.

Biovolume of several taxa within each substratum changed with stream and season, but not always consistently on the different substrata (Fig 4.7; Table 4.7). Most taxa showed significant biovolume changes for only one substratum. Biovolume of *E. maior* and cyanobacterial filaments was significantly higher during the high light month and biovolume of *E. pectinalis* v. *minor* and *M. constrictum* was significantly lower in the enriched stream only on bedrock (Table 4.7). On moss, the biovolume of both *F. rhomboides* and *G. acuminatum* v. *pusillum* were significantly lower in the enriched stream relative to the reference stream, and *G. acuminatum* v. *pusillum* biovolume was also higher during the high light month (Table 4.7). Biovolume of *G. parvulum* was significantly lower in the enriched stream on bedrock, and, being the only taxon to show significant differences in biovolume for more than one substratum, was significantly higher during the high light month on both moss and bedrock (Table 4.7). There was a significantly higher biovolume only for *M. constrictum* from the liverwort in May compared to July (Table 4.7).

Discussion

Substratum Effects

We have shown that periphytic algal assemblages can sort based on substratum. Differences in algal communities are repeatedly found between plants and geologic substrata (Pentecost 1991; Rothfritz et al. 1997; Passy et al. 1999; Lim et al. 2001). In this study, epiphytic algal communities from moss and liverwort were different from periphyton communities on bedrock, and bryophyte substrata supported several orders of magnitude higher biovolume compared to bedrock. Physical structure of the substratum may account for some of the variation in algal assemblages. The more complex configuration of bryophytes can capture colonists from the water column better than flat bedrock or tile (Burkholder and Sheath 1984; Burkholder 1996), and the much larger amount of surface area available for algal colonization would support larger quantities of algal cells. Also, the reduced shear stress and protective physical structure of bryophytes could make it less difficult for periphyton to stay established after initial colonization. Bryophytes are also leaky organisms (especially compared to the relatively inert bedrock) creating a potentially negative or positive interface with epiphytic periphyton. Evidence for allelopathy from vascular plants and the alga, *Chara*, affecting algal epiphytes in freshwater systems is equivocal (e.g. Gross et al. 2003), but there is ample evidence of vascular plants as a source of nutrients for epiphytes (Moeller et al. 1988; Burkholder and Wetzel 1990; Kahlert and Pettersson 2002). While bryophytes potentially directly stimulate their algal epiphytes, we know of no studies with bryophytes to examine potential negative or positive chemical effects of bryophytes on their epiphytes.

Bryophytes supported substantially more biomass of periphytic algae compared to bare bedrock per unit of streambed. In this study, algal biovolume was several orders of magnitude

greater on bryophytes compared to bedrock. In other studies, algal chlorophyll has also been shown to attain several fold higher levels on mosses compared to rocks (Arscott et al. 1998; Chantha et al. 2000; Slavik et al. 2004). The ability of bryophytes to support greater periphytic biomass becomes critical in consideration of stream periphyton communities in general because of how important bryophytes can be in streams. For example, about 50% of the bedrock face in our streams had some amount of bryophyte coverage (pers. obs.). Also, a significant portion of streams in many areas of the world may contain bryophytes (Slack and Glime 1985; Glime and Vitt 1987; Suren 1993; Suren 1996; Suren and Ormerod 1998; Cattaneo and Fortin 2000). Thus, epiphytes from bryophytes can clearly make up a substantial proportion of total stream periphyton. Understanding the function of bryophytes and their algal epiphytes in ecosystem processes is another important research direction.

Bryophytes showed no response to nutrient enrichment in this study. How nutrients limit aquatic bryophytes (and their potential to increase substratum available to algal colonization) is unclear since so little work has been done on this subject. Work in a tundra stream in AK showed that bryophytes responded dramatically to nutrient enrichment, although the effects were not seen for seven years (Bowden et al. 1994; Finlay and Bowden 1994; Slavik et al. 2004). It does not seem likely, however, that a longer exposure time to nutrients in this study would result in increased bryophyte biomass. For this study, the total exposure time of bryophytes to nutrients (about 730 days) was actually greater than the seven years of nutrient enrichment in the Kuparuk study which occurred for about 50 days each summer, resulting in a total exposure time to nutrient enrichment of about 350 days before bryophyte biomass increased. Also, the moss species that responded to enrichment in the Kupuruk were rare in the unenriched reach, but common in upstream seeps with naturally higher nutrient levels (Bowden et al. 1994). Thus, the

seeps served as a source of propagules of nutrient-loving bryophyte taxa. Our streams had no such source of propagules from taxa growing in higher nutrient habitats, potentially preventing increases in bryophyte biomass due to a lack of presence of a bryophyte species which could take advantage of the higher nutrient levels. There is also evidence that organic enrichment can increase bryophyte biomass (Hussey 1982; Kelly and Huntley 1987). However, work from a shaded headwater stream in TN did not show response of bryophyte biomass to nutrient enrichment (Steinman 1994). It is also possible that bryophytes may not be able to respond to nutrients under shaded conditions due to light limitation. We need a better understanding of the factors involved in bryophyte response to nutrient enrichment and the potential resulting effects on algal epiphytes.

Potential Nutrient and Light Effects

There were no positive effects of nutrient availability seen for total biovolume on any of the substrata. Establishing any differences seen between streams conclusively as nutrient effects is difficult since there are no pre-treatment data for periphyton in this study. However, algal biovolume tended to be lower in the enriched stream compared to the reference stream meaning it is likely that nutrients were not affecting biovolume accrual in these streams. Light availability, however, significantly affected algal biovolume. Although light was not manipulated per se, differences in light levels between the two sampling months were likely great enough to affect algal biomass. In a concurrent study, periphyton grown on tiles was assessed over the 3 year enrichment study (Chapter 3). Algal chlorophyll and growth rates from this study were greater during periods of high light vs. low light, consistent with results seen here of generally higher algal biovolume during the high light month. Also photosynthesis-irradiance curves of periphyton have shown the fastest increases in primary production can occur up to 100 μmol m^{-2}

s^{-1} (Vadeboncoeur and Lodge 2000) and 200 μmol m^{-2} s^{-1} (Hill et al. 1995). This suggests that productivity rates may change enough between 10 and 100 or 200 μmol m^{-2} s^{-1}, the range of light levels in the enriched and reference streams, respectively, to result in significant differences in biomass.

A pattern in biovolume regarding light availability between the streams emerged. Algal biovolume tended to be greater in the reference stream during the high light month for bedrock and moss. Also, samples with the highest measurements of algal biovolume occurred during high light months in the reference stream for moss, bedrock and tile (Chapter 3). Further, the maximum biovolume recorded from the long-term study with tiles also occurred in the reference stream at one sampling date (Chapter 3). The reference stream is perhaps generally more favorable for algal growth since light levels are slightly higher compared to the enriched stream. Alternatively, grazers may have potentially limited periphyton biomass accrual in the treatment stream as grazer biomass attained higher maxima there compared to the reference stream (Chapter 3). Grazers were also thought to play a role in limiting epiphyte response to nutrient enrichment in Walker Branch (Steinman 1994).

In addition to the differences seen across natural substrata in this study, comparisons can be made to the concurrent study in the same streams examining periphyton from tiles. Several studies have acknowledged potential discrepancies in the representation of natural communities when using artificial substrata to analyze periphyton communities (e.g. Cattaneo and Amireault 1992; Barbiero 2000; Danilov and Ekelund 2001; Lane et al. 2003). In a general comparison to taxa found on tiles (Chapter 3), there were about 2 orders of magnitude greater biovolume and 2/3 more diatom species found on tiles compared to bedrock. The higher biovolume measured on tiles was probably due to the more efficient processing that is possible by being able to remove

the tile from the stream. More diatom species were most likely found because 5 times the number of tile samples were counted compared to bedrock samples in this study. Regardless of the differences in the number of species found between tiles and bedrock, many of the filamentous algae found on natural substrata were not found on tile, which agrees with Cattaneo and Amireault (1992) who found an under representation of green algae and cyanobacteria on artificial substrata. However, all of the diatom taxa found on bedrock were also found on tile.

Responses of algal taxa to substratum, light and nutrients.

There were taxa that tended to characterize certain substrata. *G. acuminatum* var. *pusillum* and *G. parvulum* were dominant on moss relative to the other substrata. Both of these species have been recorded as common epiphytes from a stream in South Carolina (Camburn and Lowe 1978). Also, *G. parvulum* attained highest levels of relative abundance as an epiphyte in the River Mesta, Bulgaria (Passy et al. 1999). *A. subrostrata* v. *appalachiana* tended to be most common on the liverwort and formed a cobble-stone type layer on the leaflet, potentially outcompeting other taxa for colonization space. The significantly lower biovolume on bedrock for some of the taxa (*M. constrictum*, cyanobacterial filaments, *A. subrostrata* v. *appalachiana*, *E. pectinalis* v. *minor*) was probably due to the overall substantially lower biovolume on bedrock relative to the bryophytes. The taxa with no significant overall biovolume differences among the substrata (*Audouinella* sp., *A. minutissimum*, *E. maior*, *F. rhomboides*) were actually dominant on bedrock and more consistently found on bedrock relative to the bryophytes. Further, *F. rhomboides* and *Audouinella* sp. had high correlations with the NMS axes (Fig 4.5) suggesting their importance in distinguishing bedrock algal communities from bryophyte epiphytes. *E. maior* is a very large cell (about 8000 μm^3 $cell^{-1}$) so it was fairly rare in abundance but made up a large part of the biovolume, particularly on bedrock during May.

There were several taxa that were positively associated with high light environments. *A. minutissimum, F. rhomboides, G. parvulum*, and *M. constrictum* were all more abundant during higher light availability. *G. parvulum* and *M. constrictum* also showed highest relative abundance during spring in Chapter 3, *G. parvulum* and *A. minutissimum* higher in clear-cut stream at Coweeta (Lowe et al. 1986). *G. parvulum* and *M. constrictum* both showed highest abundance during high light months (Camburn and Lowe 1978). In Walker Branch, *Meridion circulare* and *G. parvulum* were most dominant from artificial substrata in the spring (Steinman and Parker 1990). *Meridion* has also been shown to prefer colder temperatures (Patrick 1971; Lowe 1974) which may explain its peak during the colder months of the year. Also, Rosemond et al. (2000) reported that *M. circulare*, a closely related taxon, was stimulated by higher light levels, indicating that this genus may also do well under higher light levels in addition to colder temperatures.

Several taxa were negatively associated with nutrients, including *A. minutissimum*, *F. rhomboides*, *G. acuminatum* v. *pusillum*, *G. parvulum* and *M. constrictum*. Except for *G. acuminatum* v. *pusillum* these taxa were all also positively associated with high light conditions. A negative association with nutrients is expected for *F. rhomboides*, a taxon that is typically found in soft-water, low-nutrient habitats (Gaiser and Johansen 2000). Also, *M. constrictum* has been found to prefer low nutrient conditions (Dixit et al. 1999). However *G. parvulum* (McCormick et al. 1996) and *A. minutissimum* (Marks and Power 2001) generally increase with nutrient enrichment. Why these taxa would increase instead of decrease with nutrient enrichment in this study is unclear. Also, there were no taxa that were clearly associated with either the enriched or the reference stream, which was consistent with the long-term study using tiles

where only one taxon, *Navicula tantula*, which was rarely present in the periphyton from this study, showed a preference for the enriched stream (Chapter 3).

Taxa were inconsistent in their response to nutrient or light availability across substrata. Only *G. parvulum* biovolume showed positive effects of light availability from two substrata (moss and bedrock). There were significant effects on *F. rhomboides* (stream) and *G. acuminatum* v. *pusillum* (stream and month) on moss, while the only changes in *E. maior* (month), *E. pectinalis* v. *minor* (stream), *M. constrictum* (stream) and cyanobacterial filaments (light) were from bedrock. Unlike taxa from moss or bedrock, taxa associated with the liverwort did not change at all with environmental variation. Also, no clear pattern emerged to indicate whether nutrient or light availability was more influential on any particular substratum. Thus, algal taxa seem to respond differently to environmental variation depending on substratum, but mechanisms driving the differences are unclear.

In conclusion, the bryophyte-algal epiphyte complex has the potential to significantly contribute to total stream periphyton, as the area of bryophyte available for algal colonization can be several orders of magnitude greater that bare rock. Also, periphytic algal assemblages can show differences across substrata in headwater streams. In our heavily shaded system, however, we saw more of an effect of light availability compared to nutrient enrichment in the accrual of algal biomass. While there were taxon-specific responses regarding light (generally positive) or nutrient (generally negative) availability, and these differences were not consistent across substrata, and no clear pattern emerged to explain those differences. Thus, variability in substratum types in headwater streams appears to support a greater diversity of periphyton and response to environmental change than would be observed from a single substratum. Clearly, all

dominant substrata need to be considered when assessing affects of environmental variation on periphyton from headwater streams.

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References

Albay, M. and R. Akcaalan. 2003. Compariative study of periphyton colonisation on common reed (*Phragmites australis*) and artificial substrate in a shallow lake, Manyas, Turkey. Hydrobiologia 506-509: 531-540.

Arscott, D. B., W. B. Bowden and J. C. Finlay. 1998. Comparison of epilithic algal and bryophyte metabolism in an arctic tundra stream, Alaska. Journal of the North American Benthological Society 17: 210-227.

Barbiero, R. P. 2000. A multi-lake comparison of epilithic diatom communities on natural and artifical substrates. Hydrobiologia 438: 157-170.

Borchardt, M. A. 1996. Nutrients. *In* Algal ecology: freshwater benthic ecosystems. R. J. Stevenson, M. L. Bothwell and R. L. Lowe, eds. San Diego, Academic Press. pp. 183-227.

Bowden, W. B., J. C. Finlay and P. E. Maloney. 1994. Long-term effects of PO_4 fertilization on the distribution of bryophytes in an arctic river. Freshwater Biology 32: 445-454.

Burkholder, J. M. 1996. Interactions of Benthic Algae with Their Substrata. *In* Algal ecology: freshwater benthic ecosystems. R. J. Stevenson, M. L. Bothwell and R. L. Lowe, eds. San Diego, Academic Press. pp. 253-297.

Burkholder, J. M. and R. G. Sheath. 1984. The seasonal distribution, abundance and diversity of desmids (Chlorophyta) in a softwater, north temperate stream. Journal of Phycology 20: 159-172.

Burkholder, J. M. and R. G. Wetzel. 1990. Epiphytic alkaline phosphatase on natural and artificial plants in an oligotrophic lake: Re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. Limnology and Oceanography 35: 736-747.

Camburn, K. E. and R. L. Lowe. 1978. The Haptobenthic Diatom Flora of Long Branch Creek, South Carolina. Nova Hedwigia 30: 149-279.

Carpenter, S. R., T. M. Frost, D. Heisey and T. K. Kratz. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology 70: 1142-1152.

Carr, J. M., G. L. Hergenrader and J. N.H. Troelstrup. 1986. A Simple Inexpensive Method for Cleaning Diatoms. Transactions of the American Microscopial Society 105: 152-157.

Cattaneo, A. and M. C. Amireault. 1992. How artificial are artificial substrata for periphyton? Journal of the North American Benthological Society 11: 244-256.

Cattaneo, A. and L. Fortin. 2000. Moss distribution in streams of the Quebec Laurentian Mountains. Canadian Journal of Botany 78: 748-752.

Chantha, S.-C., L. Cloutier and A. Cattaneo. 2000. Epiphytic algae and invertebrates on aquatic mosses in a Quebec stream. Archiv für Hydrobiologie 147: 143-160.

Coleman, V. L. and J. M. Burkholder. 1994. Community structure and productivity of epiphytic microalgae on eelgrass (*Zostera marina* L.) under water-column nitrate enrichment. Journal of Experimental Marine Biology and Ecology 179: 29-48.

Coleman, V. L. and J. M. Burkholder. 1995. Response of microalgal epiphyte communities to nitrate enrichment in an eelgrass (*Zostera marina*) meadow. Journal of Phycology 31: 36-43.

Danilov, R. A. and N. G. A. Ekelund. 2001. Comparison of usefulness of three types of artificial substrata (glass, wood and plastic) when studying settlement patterns of periphyton in lakes of different trophic status. Journal of Microbiological Methods 45: 167-170.

Dixit, S.S., J.P. Smol, D.F. Charles, R.M. Hughes, S.G. Paulsen and G.B. Collins. 1999. Assessing water quality changes in the lakes of the northeastern United States using sediment diatoms. Canadian Journal of Fisheries and Aquatic Sciences 56: 131-152.

Douglas, B. 1958. The ecology of the attached diatoms and other algae in a small stony stream. Journal of Ecology 46: 295-322.

Duncan, S. W. and D. W. Blinn. 1989. Importance of physical variables on the seasonal dynamics of epilithic algae in a highly shaded canyon stream. Journal of Phycology 25: 455-461.

Finlay, J. C. and W. B. Bowden. 1994. Controls on production of bryophytes in an arctic tundra stream. Freshwater Biology 32: 455-466.

Gaiser, E.E. and J. Johansen. 2000. Freshwater diatoms from Carolina bays and other isolated wetlands on the Atlantic Coastal Plain of South Carolina, USA, with descriptions of seven taxa new to science. Diatom Research 15: 75-130.

Glime, J. M. and D. H. Vitt. 1987. A comparison of bryophyte species diversity and niche structure of montane streams and stream banks. Canadian Journal of Botany 65: 1824-1837.

Gross, E. M., C. Feldbaum and A. Graf. 2003. Epiphyte biomass and elemental composition on submersed macrophytes in shallow eutrophic lakes. Hydrobiologia 506-509: 559-565.

Hill, W. R. and A. W. Knight. 1988. Nutrient and light limitation of algae in two northern California streams. Journal of Phycology 24: 125-132.

Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollingher and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403-424.

Hussey, B. 1982. Moss growth of filter beds. Water Research 16: 391-398.

Kahlert, M. and K. Pettersson. 2002. The impact of substrate and lake trophy on the biomass and nutrient status of benthic algae. Hydrobiologia 489: 161-169.

Kelly, M. G. and B. Huntley. 1987. *Amblystegium riparium* in brewery effluent channels. Journal of Bryology 14: 792.

Lalonde, S. and J. A. Downing. 1991. Epiphyton biomass is related to lake trophic status, depth, and macrophyte architecture. Canadian Journal of Fisheries and Aquatic Sciences 48: 2285-2291.

Lane, C. M., K. H. Taffs and J. L. Corfield. 2003. A comparison of diatom community structure on natural and artificial substrata. Hydrobiologia 493: 65-79.

Lim, D. S. S., C. Kwan and M. S. V. Douglas. 2001. Periphyton diatom assemblages from Bathurst Island, Nanavut, Canadian High Arctic: an examination of community relationships and habitat preferences. Journal of Phycology 37: 379-392.

Lohman, K., J. R. Jones and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. Journal of the North American Benthological Society 10: 14-23.

Lowe, R. L. 1974. Environmental Requirements and Pollution Tolerance of Freshwater Diatoms. 640/4-74-005, USEPA, Cincinnati, Ohio**.**

Lowe, R. L., S. W. Golladay and J. R. Webster. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. Journal of the North American Benthological Society 5: 221-229.

Marks, J.C. and M.E. Power. 2001. Nutrient induced changes in the species composition of epiphytes on *Cladophora glomerata* Kütz. (Chlorophyta). Hydrobiologia 450: 187-196.

McCormick, P.V., P.S. Rawlik, K. Lurding, E.P. Smith, and F.H. Sklar. 1996. Periphyton-water quality relationships along a nutrient gradient in the northern Florida Everglades. Journal of the North American Benthological Society 15: 422-449.

McCune, B. and J. B. Grace 2002. Analysis of Ecological Communities. MjM Software Design. Gleneden Beach, Oregon. 300 pp.

Minchin, P. R. 1987. An evaluation of the relative robustness of techniques for ecological ordination. Vegetatio 69: 89-107.

Moeller, R. E., J. M. Burkholder and R. G. Wetzel. 1988. Significance of sedimentary phosphorus to a footed submersed macrophyte (*Najas flexilis* (Willd.) Rostk. and Schmidt) and its algal epiphytes. Aquatic Botany 32: 261-281.

Mulholland, P. J. and A. D. Rosemond. 1992. Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream-downstream linkage. Journal of the North American Benthological Society 11: 405-419.

Naiman, R. J. 1983. The annual pattern and spatial distribution of aquatic oxygen metabolism in boreal forest watersheds. Ecological Monographs 53: 73-94.

Neckles, H. A., R. L. Wetzel and R. J. Orth. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. Oecologia 93: 285-295.

Neundorfer, J. V. and W. M. Kemp. 1993. Nitrogen versus phosphorus enrichment of brackish waters: responses of the submersed plant *Potamogeton perfoliatus* and its associated algal community. Marine Ecology Progress Series 94: 71-82.

Passy, S. I., Y. Pan and R. L. Lowe. 1999. Ecology of the major periphytic diatom communities from the Mesta River, Bulgaria. International Review of Hydrobiology 84: 129-174.

Patrick, R. 1971. The effects of increasing light and temperature on the structure of diatom communities. Limnology and Oceanography 16: 405-421.

Pentecost, A. 1991. Algal and bryophyte flora of a Yorkshire (U.K.) hill stream: a comparative approach using biovolume estimations. Archiv für Hydrobiologie 121: 181-201.

Pentecost, A. 1998. The significance of calcite (travertine) formation by algae in a moss-dominated travertine from Matlock Bath, England. Archiv für Hydrobiologie 143: 487-509.

Rier, S. T. and R. J. Stevenson. 2002. Effects of light, dissolved organic carbon, and inorganic nutrients on the relationship between algae and heterotrophic bacteria. Hydrobiologia 489: 179-184.

Rosemond, A. D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia 94: 585-594.

Rosemond, A. D. 1994. Multiple factors limit seasonal variation in periphyton in a forest stream. Journal of the North American Benthological Society 13: 333-344.

Rosemond, A. D., P. J. Mulholland and S. H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Canadian Journal of Fisheries and Aquatic Sciences 57: 66-75.

Rosemond, A. D., P. J. Mulholland and J. W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74: 1264-1280.

Rothfritz, H., I. Jüttner, A. M. Suren and S. J. Ormerod. 1997. Epiphytic and epilithic diatom communities along environmental gradients in the Nepalese Himalaya: implications for the assessment of biodiversity and water quality. Archiv für Hydrobiologie 138: 465-482.

Scott, M. C., G. S. Helfman, M. E. McTammany, E. F. Benfield and P. Bolstad, V. 2003. Multiscale influences on physical and chemical stream conditions across Blue Ridge landscapes. Journal of the American Water Resources Association 38: 1379-1392.

Slack, N. G. and J. M. Glime. 1985. Niche relationships of mountain stream bryophytes. The Bryologist 88: 7-18.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River Ecosystem to phosphorus fertilization. Ecology 85: 939-954.

Steinman, A. D. 1994. The influence of phosphorus enrichment on lotic bryophytes. Freshwater Biology 31: 53-63.

Steinman, A. D. and H. L. Boston. 1993. The ecological role of aquatic bryophytes in a woodland stream. Journal of the North American Benthological Society 12: 17-26.

Steinman, A. D. and A. F. Parker. 1990. Influence of substrate conditioning on periphytic growth in a hetertrophic woodland stream. Journal of the North American Benthological Society 9: 170-179.

Stevenson, R. J., M. L. Bothwell and R. L. Lowe, Eds. 1996. Algal ecology: freshwater benthic ecosystems. Aquatic Ecology. San Diego, Academic Press.

Stream Bryophyte Group. 1999. Roles of bryophytes in stream ecosystems. Journal of the North American Benthological Society 18: 151-184.

Suren, A. M. 1992. Enhancement of invertebrate food resources by bryophytes in New Zealand alpine headwater streams. New Zealand Journal of Marine and Freshwater Research 26: 229-239.

Suren, A. M. 1992. The influence of periphyton, detritus and shelter on invertebrate colonization of aquatic bryophytes. Freshwater Biology 27: 327-339.

Suren, A. M. 1993. Bryophytes and associated invertebrates in first-order alpine streams of Arthur's Pass, New Zealand. New Zealand Journal of Marine and Freshwater Research 27: 479-494.

Suren, A. M. 1996. Bryophyte distribution patterns in relation to macro-, meso-, and micro-scale variables in South Island, New Zealand streams. New Zealand Journal of Marine and Freshwater Research 30: 501-523.

Suren, A. M. and S. J. Ormerod. 1998. Aquatic bryophytes in Himalayan streams: testing a distribution model in a highly heterogeneous environment. Freshwater Biology 40: 697-716.

Swank, W. T. and D. A. Crossley 1988. Forest Hydrology and Ecology at Coweeta. Springer-Verlag. New York. 469 pp.

Tank, J. L. and W. K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48: 1031-1049.

Vadeboncoeur, Y. and D. M. Lodge. 2000. Periphyton production on wood and sediment: substratum-specific response to laboratory and whole-lake nutrient manipulation. Journal of the North American Benthological Society 19: 68-81.

Welch, E. B., J. M. Jacoby, R. R. Horner and M. R. Seeley. 1988. Nuisance biomass levels of periphytic algae in streams. Hydrobiologia 157: 161-168.

Table 4.1. Physical and chemical characteristics of the 2 reference streams, Catchments 56 and 53, and the enriched stream, Catchment 54. Discharge, and temperature are from July 1999-July 2002. Nutrient data in 53 and 54 and pH data from all streams are from the pretreatment period July 1999- July 2000. Nutrient data from WS 56 are from Jan 1993 – Dec 1995.

		Biomass Reference (C 56)	Species Reference (C 53)	Enriched (C 54)
Catchment	Area (ha)	7.5[a]	5.2	5.5
	Elevation (m a.s.l.)	810[a]	820	841
Channel	Gradient (cm m^{-1})	20[a]	27	33
	Length (m)	170[a]	145	282
	Bankfull area (m^2)	373[a]	327	443
Temperature (°C)	Daily mean (*n*)	11.7 (1127)	12.0 (336)	12.0 (336)
	Range	0-19.1	2.6-18.6	4.8-16.7
Discharge (L s^{-1})	Daily mean (*n*)	0.64 (1069)	0.32 (1114)	0.53 (1114)
	Range	0-20.2	0.006-3.8	0.06-4.8
pH	Mean (*n*)	6.62 (22)	6.59 (24)	6.87 (18)
	Range	6.14-6.99	6.2-7.0	6.6-7.9
NO_3-N (μg L^{-1})	Mean (*n*)	7.0 (45)	15.4 (5)	18.8 (12)
	Range	1.0-2.2	9.4-25.8	4.0-39.5
NH_4-N (μg L^{-1})	Mean (*n*)	3.0 (45)	9.4 (4)	9.9 (12)
	Range	1.0-1.7	0-30.4	0-24.9
SRP (μg L^{-1})	Mean (*n*)	2.0 (68)	7.6 (5)	8.8 (12)
	Range	0-42.0	0-20.3	0-22.1

Table 4.2. Average pretreatment (July 1999 – July 2000) and treatment (July 2000 – July 2002) nutrient levels (μg L^{-1}) for the reference (C 53) and treatment (C 54) streams. Numbers in parentheses are one standard deviation. BD = below detection.

Site	$(NO_2^-+NO_3^-)$-N	$NH4^+$-N	SRP	Molar DIN:SRP
Pretreatment				
Reference				
Mean n=5	15.4 (6.6)	9.4 (14.1)	7.6 (8.0)	7.2
Range	9-26	BD-30	BD-20	
Treatment				
Mean n=12	18.8 (11.5)	9.9 (8.6)	8.8 (8.1)	7.2
Range	4-40	BD-25	BD-22	
Treatment				
Reference				
Mean n=33	16.9 (29.8)	10.4 (16.9)	3.7 (4.7)	16.3
Range	BD-151	BD-76	BD-17	
Treatment				
Mean n=44	308.9 (377.8)	105.5 (119.7)	51.2 (55.6)	17.9
Range	11-1711	6-566	BD-268	

Table 4.3. F-values from 3-way ANOVA of log total biovolume. Multiple comparison tests were conducted for significant effects of substratum (M=moss, L=liverwort and BR=bedrock) with a Tukey test. Significant effects of season or stream indicated by (+), meaning higher values occurred in the enriched stream or during the high light month and (-), meaning lower values occurred in the reference stream or low light month.

Treatment	df	Total Biovolume
Substratum	2	247.07 ****
Multiple Comparison		M+L>BR
Stream	1	ns
Season	1	(+) 6.32 *
Substrate * Stream	1	ns
Substrate * Month	2	ns
Stream * Month	1	ns
Substrate* Stream *Month	2	3.13 §

§ $p \leq 0.10$, *$p \leq 0.05$, **** $p \leq 0.0001$

Table 4.4. Average cell biovolume (μm^3 cm^{-2} bedrock) and standard deviations of all cells and algal divisions for moss, liverwort and bedrock in the reference and treatment streams during May (high light) and July (low light).

Substrate	Stream	Month		Total	Diatoms	Chrysophyta	Chlorophyta	Cyanobacteria	Rhodophyta
Moss	Ref	May	Mean	1.95×10^8	1.93×10^8	0	3.86×10^5	1.4×10^6	2039
			(SD)	(2.58×10^8)	(2.57×10^8)	(0)	(2.14×10^5)	(9.51×10^5)	(3,531)
		July	Mean	1.43×10^7	1.39×10^7	459	11,454	403,229	8,687
			(SD)	(7.89×10^6)	(7.98×10^6)	(796)	(9,938)	(1.83×10^5)	(6,951)
	Tmt	May	Mean	2.22×10^7	2.21×10^7	0	0	1.23×10^5	1,399
			(SD)	(2.14×10^7)	(2.14×10^7)	(0)	(0)	(68,776)	(2,424)
		July	Mean	1.40×10^7	1.32×10^7	0	0	8.44×10^5	0
			(SD)	(1.20×10^7)	(1.12×10^7)	(0)	(0)	(8.47×10^5)	(0)
Liverwort	Ref	May	Mean	8.70×10^7	8.48×10^7	0	2.1×10^6	86,415	36,747
			(SD)	(1.27×10^8)	(1.24×10^8)	(0)	(3.6×10^6)	(90,266)	(63,523)
		July	Mean	4.13×10^7	4.13×10^7	25	137	10,761	17
			(SD)	(3.49×10^7)	(3.49×10^7)	(44)	(119)	(18,137)	(30)
	Tmt	May	Mean	2.95×10^8	2.90×10^8	55	0	5.30×10^6	0
			(SD)	($2.13 \times 10^{8)}$)	(2.21×10^8)	(96)	(0)	(8.95×10^6)	(0)
		July	Mean	2.21×10^7	2.17×10^7	0	0	368,981	45
			(SD)	($1.97 \times 10^{7)}$)	(1.93×10^7)	(0)	(0)	(629,615)	(78)

Table 4.4, cont'd.

Substrate	Stream	Month		Total	Diatom	Chrysophyta	Chlorophyta	Cyanobacteria	Rhodophyta
Bedrock	Ref	May	Mean	51,770	48,546	0	0	1,619	1,606
			(SD)	(47,692)	(45,530)	(0)	(0)	(1,136)	(1,585)
		July	Mean	15,904	13,892	0	0	922	1,089
			(SD)	(7,230)	(5,917)	(0)	(0)	(910)	(911)
	Tmt	May	Mean	30,078	26,652	0	22	3,053	351
			(SD)	(17,512)	(19,156)	(0)	(38)	(2,008)	(218)
		July	Mean	8,208	6,027	0	0	225	1,956
			(SD)	(2,884)	(2,930)	(0)	(0)	(251)	(789)

Table 4.5. Algal taxa encountered from periphyton samples on moss, liverwort and bedrock. Categories based on average relative biovolume across all samples for that substratum : A = abundant (>20%), C = common (5-20%), U = uncommon (1-5%), R = rare (<1%) of relative biovolume. A dash (-) indicates that the species was not detected from that substratum. Species in bold were analyzed for effects of substratum and nutrient and light availability.

	Moss	Liverwort	Bedrock
BACILLARIOPHYTA			
Achnanthes stewartii Patr.	R	R	-
Achnanthes subrostrata* var. *appalachiana Camburn and Lowe	A	A	C
Achnanthidium minutissimum (Kutz.) Czarn.	R	R	U
Cymbella tumida (Breb ex Kuetz.)	U	U	R
Diatoma hiemale var. *mesodon* (Ehr.) Grun.	R	R	-
Encyonema minutum (Hilse) Mann	R	R	R
Eunotia maior (W. Sm.) Rabh.	C	C	A
Eunotia pectinalis* var. *minor (Kuetz.) Rabh.	C	C	A
Eunotia pectinalis var. *recta* A. Mayer ex Patr.	C	R	-
Frustulia rhomboides (Ehr.) De T.	U	U	C
Gomphonema acuminatum* var. *pusillum Grun.	C	R	-
Gomphonema parvulum (Kuetz.)	C	R	U
Meridion constrictum Ralfs	C	A	C
Navicula angusta Grun.	U	R	U
Navicula placenta Ehr.	R	R	-
Navicula tantula Hust.	U	R	-
Nitzschia palea (Kuetz.) W. Sm.	R	R	R
Pinnularia mesogongyla Ehr. var. *mesogongyla*	R	R	-
CHRYSOPHYTA			
Unidentified stomatocysts	R	R	-
CHLOROPHYTA			
Cosmarium spp.	R	R	-
Mougeotia sp.	R	R	-
Oedogonium sp.	-	R	R
RHODOPHYTA			
Audouinella sp.	R	R	C

Table 4.5 (cont'd).

	Moss	Liverwort	Bedrock
CYANOBACTERIA			
Anabaena sp.	-	R	-
Chamaesiphon sp.	R	R	R
Coccoid cyanobacteria	R	R	-
Oscillatoria spp.	U	U	U
Tolypothrix sp.	R	R	-
Other filamentous cyanobacteria	R	-	U
Total Taxa Richness	27	28	16

Table 4.6. F-values from 3-way ANOVA of log absolute biovolume of different algal species. Multiple comparison tests were conducted for significant effects of substratum (M=moss, L=liverwort and BR=bedrock) with a Tukey test. Significant effects of season or stream indicated by (+), meaning higher values occurred in the enriched stream or during the high light month and (-), meaning higher values occurred in the reference stream or low light month. ACHAPP = *Achnanthes subrostrata* var. *appalachiana*, ACHMIN = *Achnanthidium minutissimum*, EUNMAI = *Eunotia maior*, EUPECM = *Eunotia pectinalis* var. *major*, FRURHO = *Frustulia rhomboides*, GOMACU = *Gomphonema acuminatum*, GOMPAR = *Gomphonema parvulum*, MERCON = *Meridion constrictum*, CYFIL = cyanobacterial filaments, AUDOUI = *Audouinella* sp.

Treatment	df	ACHAPP	ACHMIN	EUNMAI	EUPECM	FRURHO
Substratum	2	122.69 ****	ns	ns	152.14 ****	ns
Multiple Comparison		M=L>BR			M=L>BR	
Stream	1	ns	(-) 17.67 ***	ns	ns	(-) 5.04 *
Month	1	ns	(+) 4.42 *	ns	ns	(+) 3.33 §
Substrate * Stream	1	ns	10.04 ***	ns	ns	3.43 *
Substrate * Month	2	ns	ns	ns	ns	ns
Stream * Month	1	ns	ns	ns	ns	ns
Substrate* Stream * Month	2	ns	ns	ns	ns	ns

§ $p \leq 0.10$, *$p \leq 0.05$, ***$p \leq 0.001$, **** $p \leq 0.0001$

Table 4.6, (cont'd).

Treatment	df	GOMACU	GOMPAR	MERCON	CYFIL	AUDOUI
Substratum	2	15.55 ****	231.82****	41.69 ****	27.03****	ns
Multiple Comparison		M>L=BR	M>L>BR		M>L>BR	
Stream	1	(-) 5.19 *	(-) 8.51 **	(-) 5.15 *	ns	ns
Month	1	ns	(+) 21.44 ****	(+)6.20 *	ns	ns
Substrate * Stream	1	6.39 **	ns	ns	ns	ns
Substrate * Month	2	ns	ns	ns	ns	ns
Stream * Month	1	ns	ns	ns	ns	ns
Substrate* Stream * Month	2	ns	ns	ns	ns	ns

*$p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$

Table 4.7. F-values from 2-way ANOVA of log-transformed biovolume of different algal species. Significant effects of season or stream indicated by (+), meaning higher values occurred in the enriched stream or during the high light month and (-), meaning lower values occurred in the reference stream or low light month. A dash (-) indicates the species was not common enough on that substratum for analysis. Species abbreviations: ACHAPP = *Achnanthes subrostrata* var. *appalachiana*, ACHMIN = *Achnanthidium minutissimum*, EUNMAI = *Eunotia maior*, EUPECM = *Eunotia pectinalis* var. *major*, FRURHO = *Frustulia rhomboides*, GOMACU = *Gomphonema acuminatum*, GOMPAR = *Gomphonema parvulum*, MERCON = *Meridion constrictum*, CYFIL = cyanobacterial filaments, AUDOUI = *Audouinella* sp.

Treatment	df	ACHAPP	ACHMIN	EUNMAI	EUPECM	FRURHO
MOSS						
Stream	1	ns	-	ns	ns	(-) 9.42 *
Month	1	ns	-	ns	ns	ns
Stream * Month	1	ns	-	ns	3.46 §	ns
LIVERWORT						
Stream	1	ns	-	ns	ns	ns
Month	1	ns	-	ns	ns	ns
Stream * Month	1	ns	-	ns	ns	ns
BEDROCK						
Stream	1	ns	ns	ns	(-) 29.55 **	ns
Month	1	ns	ns	(+) 8.66 *	ns	ns
Stream * Month	1	ns	ns	ns	ns	ns

§ $p \leq 0.10$, *$p \leq 0.05$, ** $p \leq 0.01$

Table 4.7 (cont'd).

Treatment	df	GOMACU	GOMPAR	MERCON	CY FIL	AUDOUI
MOSS						
Stream	1	(-) 20.89 **	ns	ns	-	-
Month	1	(+) 5.37 *	(+) 7.79 *	ns	-	-
Stream * Month	1	ns	ns	ns	-	-
LIVERWORT						
Stream	1	-	-	ns	-	-
Month	1	-	-	(+) 9.90 *	-	-
Stream * Month	1	-	-	ns	-	-
BEDROCK						
Stream	1	-	(-) 23.82 **	(-) 3.85 §	ns	ns
Month	1	-	(+) 21.64 **	ns	(+) 8.61 *	ns
Stream * Month	1	-	10.47*	ns	ns	ns

§ $p \leq 0.10$, *$p \leq 0.05$, ** $p \leq 0.01$

Figure 4.1 Non-metric multidimensional scaling ordination of sites in species space. The three letters in the legend code indicate first substratum (M=moss, L=liverwort, B=bedrock), then month sampled (M=May (high light), J=July (low light)), then the stream sampled (R=reference, T=treatment (enriched)). Further, communities from each substratum (moss, liverwort and bedrock) are grouped separately. The amount of variance explained by each axis is in parentheses.

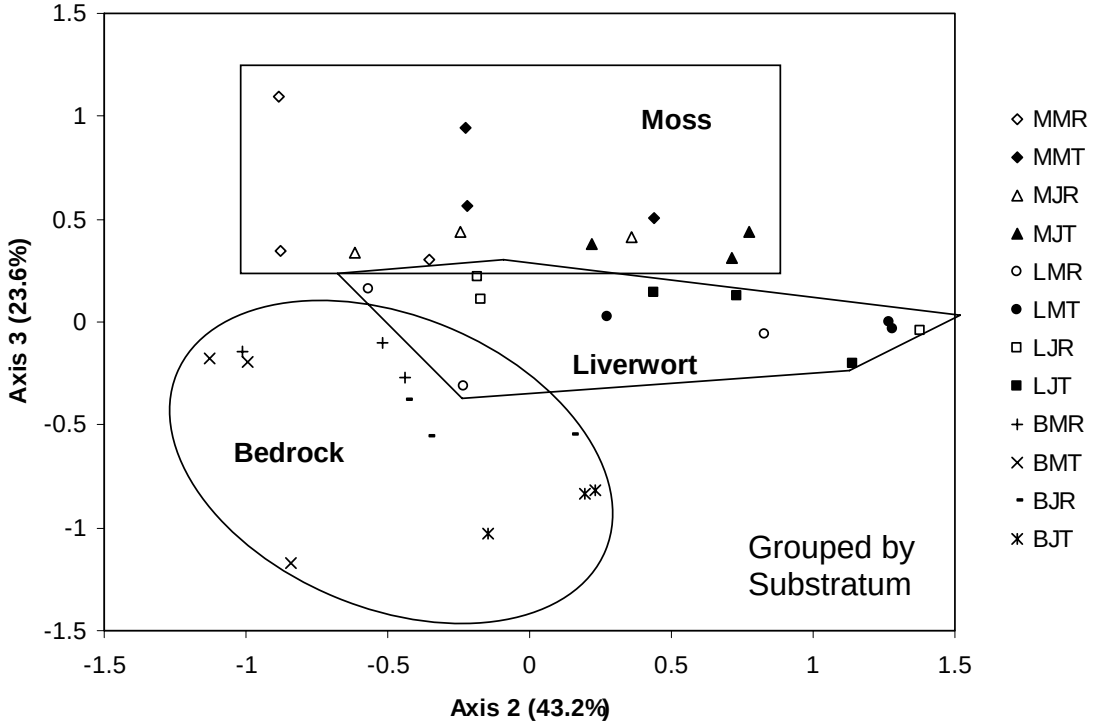

Moss
Liverwort
Bedrock
Grouped by Substratum
Axis 3 (23.6%)
Axis 2 (43.2%)
1.5
1
0.5
0
-0.5
-1
-1.5
MMR
MMT
MJR
MJT
LMR
LMT
LJR
LJT
BMR
BMT
BJR
BJT

Figure 4.2. Bubble plots of algal species in species space. The location of each symbol corresponds with the ordination plot in Fig 4.4 and represents the relative biovolume of the algal species at each site. The correlation coefficient (Pearson's *r*) for each species is shown on the axis with the highest *r* in each plot. For *Meridion constrictum*, the highest Pearson's r was for Axis 1.

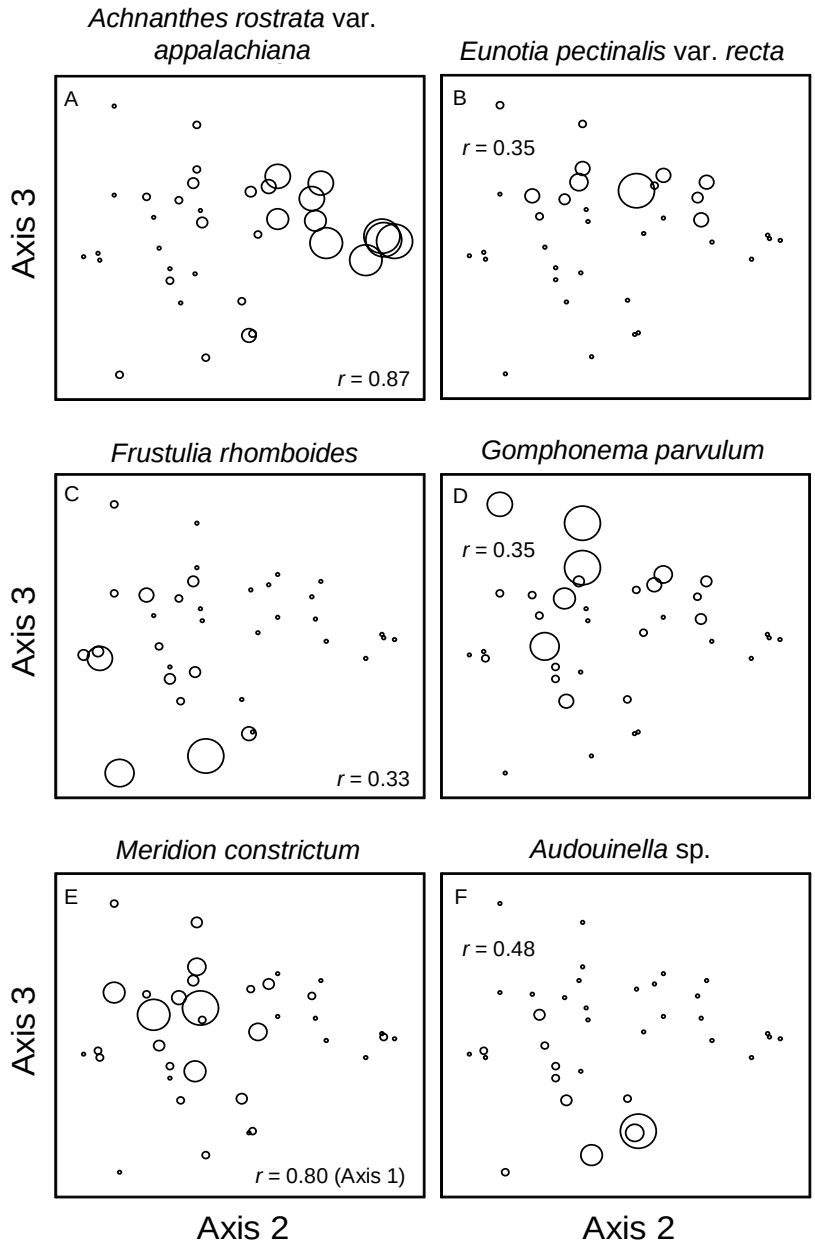

Achnanthes rostrata var. *appalachiana*
Eunotia pectinalis var. *recta*
A
B
r = 0.35
r = 0.87
Frustulia rhomboides
Gomphonema parvulum
C
D
r = 0.35
r = 0.33
Meridion constrictum
Audouinella sp.
E
F
r = 0.48
r = 0.80 (Axis 1)
Axis 3
Axis 3
Axis 3
Axis 2
Axis 2

Figure 4.3. Bryophyte AFDM ± 1 SE in the treatment and reference (C 56) streams. P >0.05 from RIA on differences between streams before and after enrichment. Arrow indicates start of nutrient enrichment. The dashed line and open symbols represent the reference stream (C 56) and the solid line and symbols represent the enriched stream (C 54).

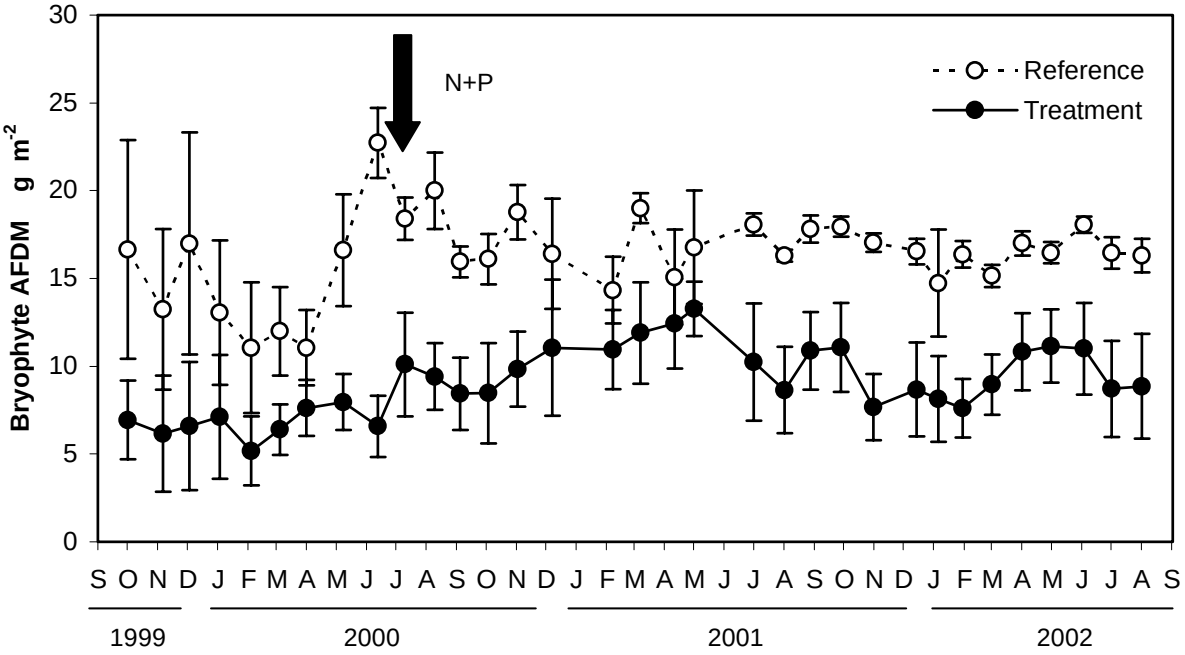

Bryophyte AFDM g m-2
30
25
20
15
10
5
0
N+P
Reference
Treatment
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S
1999
2000
2001
2002

Figure 4.4 Total algal biovolume ± 1 SE on moss (A), liverwort (B) and bedrock (C) during periods of high light (May) and low light (July) standardized for stream bed (bedrock) area. Open bars represent the reference stream (C 53) and closed bars represent the enriched stream (C 54).

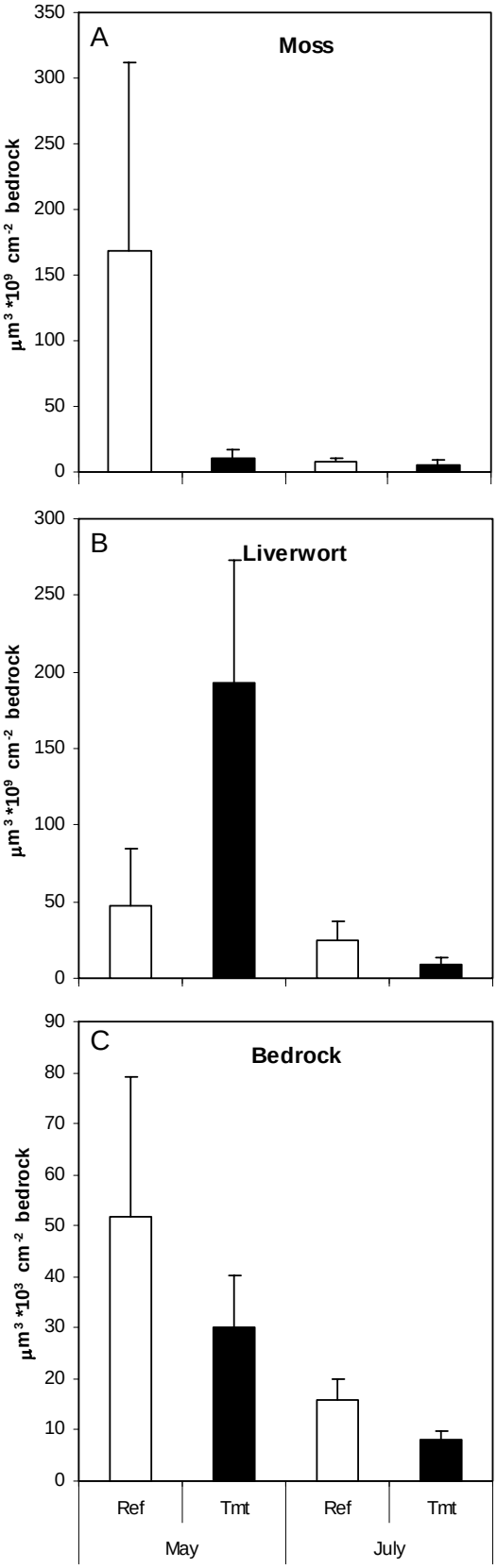

A
Moss
B
Liverwort
C
Bedrock
µm³ *10⁹ cm⁻² bedrock
µm³ *10³ cm⁻² bedrock
Ref
Tmt
Ref
Tmt
May
July

Figure 4.5 Non-metric multidimensional scaling ordination of sites in species space. The three letters in the legend code indicate first substratum (M=moss, L=liverwort, B=bedrock), then month sampled (M=May (high light), J=July (low light)), then the stream sampled (R=reference, T=treatment (enriched)). Further, data points are grouped separately by substratum and stream. Points surrounded by dashed lines are from the reference stream, and points surrounded by solid lines are from the enriched stream. The amount of variance explained by each axis is in parentheses.

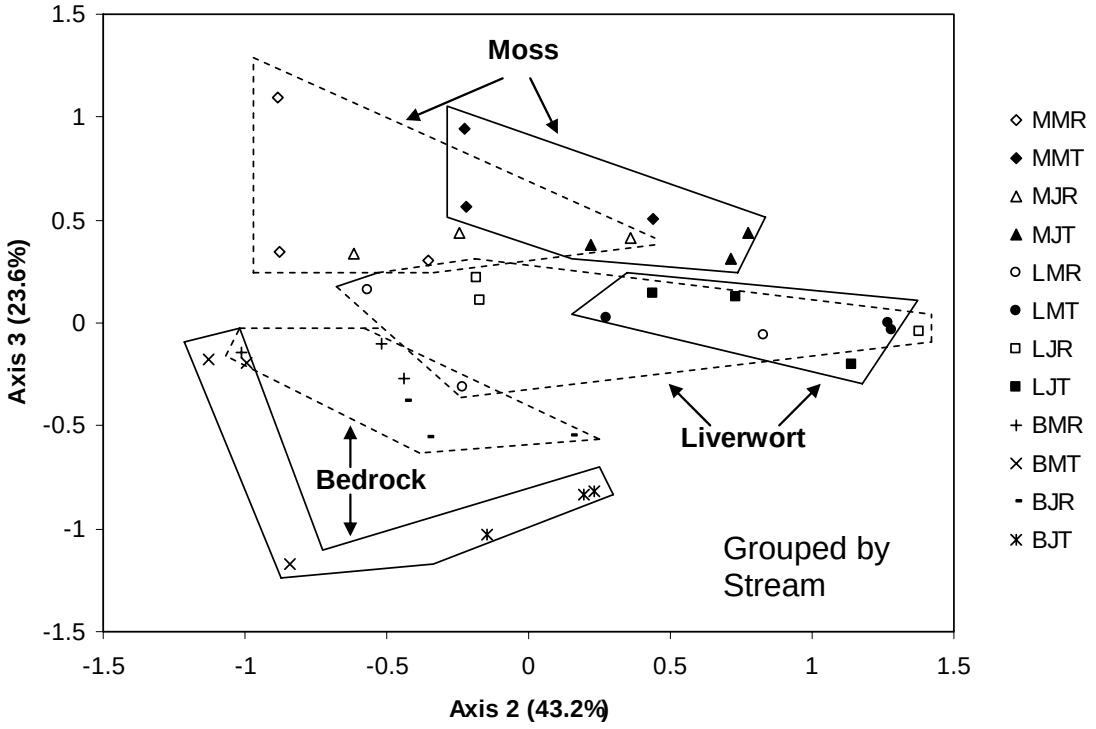

Moss
Liverwort
Bedrock
Grouped by Stream
Axis 3 (23.6%)
Axis 2 (43.2%)
1.5
1
0.5
0
-0.5
-1
-1.5
-1.5
-1
-0.5
0
0.5
1
1.5
MMR
MMT
MJR
MJT
LMR
LMT
LJR
LJT
BMR
BMT
BJR
BJT

Figure 4.6 Non-metric multidimensional scaling ordination of sites in species space. The three letters in the legend code indicate first substratum (M=moss, L=liverwort, B=bedrock), then month sampled (M=May (high light), J=July (low light)), then the stream sampled (R=reference, T=treatment (enriched)). Further, data points are grouped separately by substratum and season. Points surrounded by dashed lines are from the high light month, and points surrounded by solid lines are from the low light month. The amount of variance explained by each axis is in parentheses.

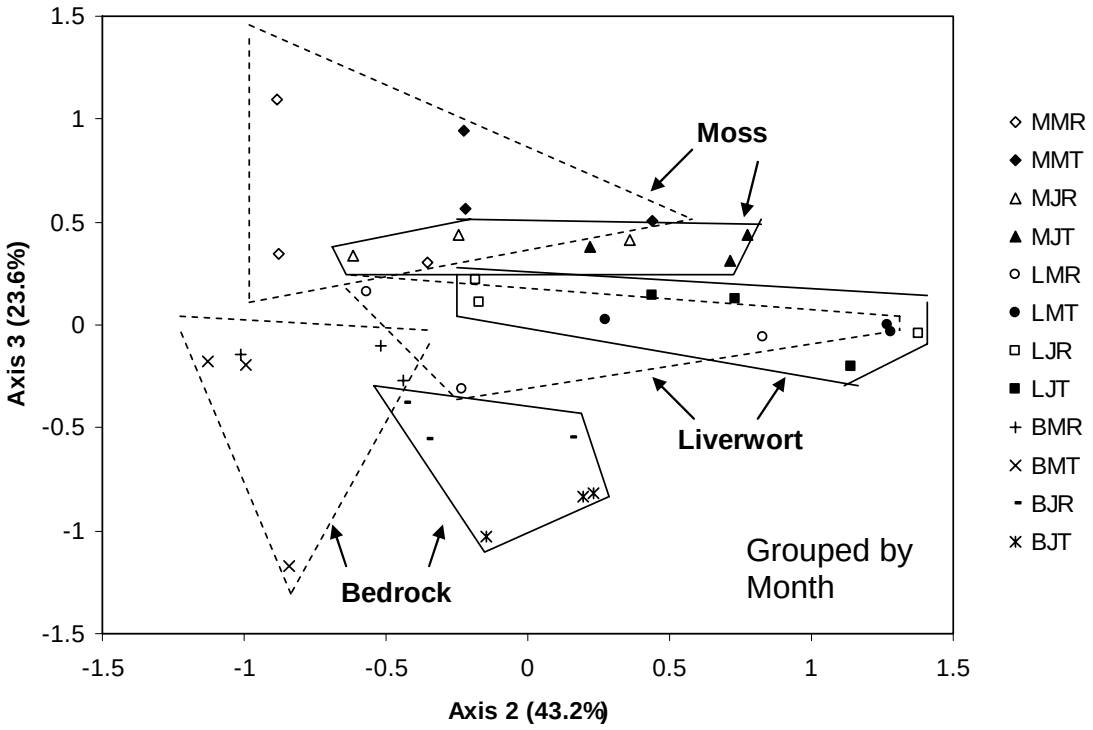
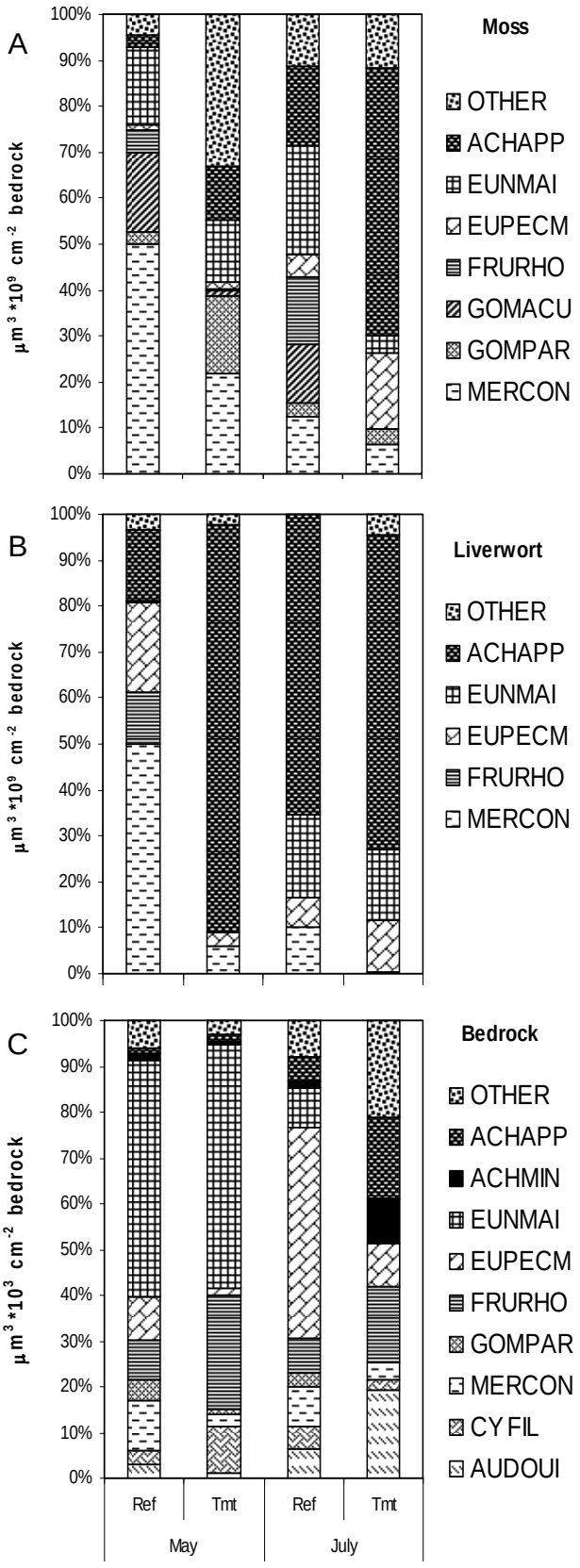

1.5
1
0.5
0
-0.5
-1
-1.5
-1.5
-1
-0.5
0
0.5
1
1.5
Axis 3 (23.6%)
Axis 2 (43.2%)
Moss
Liverwort
Bedrock
Grouped by Month
MMR
MMT
MJR
MJT
LMR
LMT
LJR
LJT
BMR
BMT
BJR
BJT

Figure 4.7. Diatom relative biovolume standardized per stream bed area for moss (A), liverwort (B) and bedrock (C). Species abbreviations: ACHAPP = *Achnanthes subrostrata* var. *appalachiana*, ACHMIN = *Achnanthidium minutissimum*, EUNMAI = *Eunotia maior*, EUPECM = *Eunotia pectinalis* var. *major*, FRURHO = *Frustulia rhomboides*, GOMACU = *Gomphonema acuminatum*, GOMPAR = *Gomphonema parvulum*, MERCON = *Meridion constrictum*, CYFIL = cyanobacterial filaments, AUDOUI = *Audouinella* sp.Fig. 4.3


A
B
C
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
µm³ *10⁹ cm⁻² bedrock
µm³ *10³ cm⁻² bedrock
Moss
Liverwort
Bedrock
OTHER
ACHAPP
ACHMIN
EUNMAI
EUPECM
FRURHO
GOMACU
GOMPAR
MERCON
CY FIL
AUDOUI
Ref
Tmt
Ref
Tmt
May
July

CHAPTER 5

CONCLUSIONS

The objectives of this dissertation were to examine the response of the detrital and autotrophic basal food web resources from a detritus-based stream to long-term nutrient enrichment. Previous short-term studies have shown that nutrient enrichment can increase processing rates of detritus (Kaushik and Hynes 1971; Howarth and Fisher 1976; Elwood et al. 1981; Meyer and Johnson 1983; Robinson and Gessner 2000; Grattan and Suberkropp 2001) and that light limitation can limit the potential autotrophic response to nutrient enrichment in these heavily shaded headwater streams (Gregory 1980; Triska et al. 1983; Lowe et al. 1986; Hill and Knight 1988; Winterbourn 1990; Rosemond 1993; Wellnitz et al. 1996; Rosemond et al. 2000; Hill et al. 2001; Mosisch et al. 2001; Tank and Dodds 2003; Bernhardt and Likens 2004). However, there have been few studies to examine the effects of long-term nutrient enrichment in detritus-based stream ecosystems (Ramírez 2001; Chadwick and Huryn 2003). Also, autotrophic components of food webs in streams heavily dependent on detritus have been rarely studied (Bernhardt and Likens 2004), and to the best of my knowledge no long-term studies have been performed.

My specific goals for this dissertation were to examine the effects of long-term nutrient enrichment on 1) breakdown rates, microbial respiration rates, C:N ratios, invertebrate communities and the amount of nitrogen contained in leaf material vs. invertebrate biomass in litter bags of a low quality leaf, rhododendron, and a higher quality leaf, red maple (Chapter 2), 2) algal biomass as ash-free dry mass and chlorophyll *a*, algal species composition, and algal

growth rates as a proxy measure of productivity (Chapter 3), and 3) the differential response in periphyton from bryophytes and epilithon and the potential role played by nutrient and light availability in structuring epiphytic algal assemblages from a detritus-based stream (Chapter 4).

In Chapter 2, I found strong effects of nutrient enrichment on detrital leaf litter. Breakdown rates of both low quality rhododendron and higher quality red maple leaves increased nearly three-fold by the end of the second year of enrichment. Increases in both microbial and invertebrate activity likely contributed to increased breakdown rates. Increased microbial respiration was measured in leaf packs for both leaf types. Presumably as a result of the increased microbial biomass, C:N levels of both leaf types were also lower in the enriched stream. The amount of invertebrate biomass from leaf packs also increased several-fold with enrichment, presumably due to the increased nutritional quality of the leaf material available. Also, the increase in breakdown rates and invertebrate biomass increased even further during the second year enrichment, suggesting that the response to nutrient enrichment had not yet reached equilibrium, and continuing studies indicate that in successive years of enrichment, breakdown rates are increasing in the treatment stream relative to the reference stream (A.D. Rosemond, unpubl. data). I have also shown that there is more stored nitrogen located in invertebrate biomass relative to leaf material with enrichment, suggesting that the efficiency and speed of nitrogen cycling through the system has increased.

In Chapter 3, I observed minimal and seasonally dependent effects of long-term nutrient enrichment on epilithic periphyton. Algal biomass increased slightly with enrichment, but only as chlorophyll *a*, and not as biovolume, suggesting a largely physiological vs. a biomass response. Growth rates were faster with enrichment, particularly during periods of high light availability. This, together with a slight increase in grazer biomass during high light availability,

suggests that herbivory may play a role in limiting algal response to nutrient enrichment. Algal species assemblages were dominated by a few species of diatoms, including *Meridion constrictum, Eunotia pectinalis* varieties *recta* and *minor*, *Gomphonema parvulum* and *Navicula tantula*, with almost no representation by non-diatom algae. The species assemblages showed more seasonal variation than any change due to nutrient enrichment. Overall, any effects that we did see required a long-term experiment which spanned several seasons of the year in order to detect a response from the periphyton.

In Chapter 4, I examined the effects of nutrient enrichment on bryophyte biomass and the response of epiphytic biomass and algal assemblages. Also, epiphyte assemblage changes in response to nutrient and light availability were compared among a common moss, *Platylomella lescurii*, a common liverwort, *Jubula pennsylvanica* and bedrock. Bryophyte biomass showed no change with enrichment, potentially due to light limitation, but also because a longer time lag may have been necessary for bryophytes to manifest a response to nutrient enrichment as an increase in biomass. Among the three substrata, bryophytes were able to support over $2x10^6$ X more biovolume compared to bedrock. If bryophytes had increased in response to nutrient enrichment, this could have had dramatic effects on the biomass of periphyton in this stream ecosystem. Periphyton communities were significantly different across all three substrata. There were significant, positive effects of light availability on total biovolume, but no effect of nutrient availability. Differences in taxon-specific response to environmental variation occurred across the three substrata, but no consistent pattern emerged to explain those differences.

Several studies occurred simultaneously with this dissertation examining other aspects of nutrient enrichment in the same streams. Together, results from this dissertation and the other studies have shown that nutrient enrichment can have profound effects on food webs in detritus-

based stream ecosystems. Bacterial, and particularly fungal, production and respiration rates from ambient leaf litter in the stream were all stimulated by nutrient enrichment (K. Suberkropp, unpublished data). Increased breakdown rates of leaf litter were seen both in this study, and another study examining mostly microbial effects on leaf litter (Gulis and Suberkropp 2003). Breakdown rates of wood were also faster (Gulis et al. 2004). Invertebrate consumers were also profoundly affected by nutrient enrichment. Nutrient, particularly phosphorus, content of invertebrate detrital consumers increased with enrichment (Cross 2004). Nutrient enrichment also stimulated consumer growth rates, invertebrate secondary production, and the total flows of organic matter to consumers. Enrichment also increased the export of fine particulate organic matter downstream and reduced leaf litter standing crop (A.D. Rosemond, unpublished data).

Ultimately, this study showed a stronger absolute response, but similar relative response of detrital leaf litter resources to nutrient enrichment compared to periphyton. Average standing crop of leaf litter in the enriched stream was roughly 1.5X lower than the reference stream during the 2 years of enrichment (K. Suberkropp, unpublished data). Similarly, average periphyton standing crop increased roughly 1.5X in the enriched stream during the enrichment period (Chapter 3). However, standing crop of leaves was over 3 orders of magnitude higher than standing crop of periphyton, about 460g leaf AFDM m^{-2} (K. Suberkropp, unpublished data) and 0.360 g periphyton AFDM m^{-2} (Chapter 3) in the reference stream and 300 g leaf AFDM m^{-2} and 0.530 g periphyton AFDM m^{-2} in the treatment stream. In sum, much more carbon was ultimately lost from the stream due to detrital processing than was gained through any increases in primary productivity.

Clearly, detrital resources can also be strongly influenced by water column nutrient concentrations which can result in significant bottom-up effects to consumers. The somewhat

limited response I saw in autotrophs to nutrient enrichment, especially compared to the strong effects of detrital resources, shows that a stream ecosystem can be fundamentally changed by nutrient enrichment without showing signs of increased autotrophic productivity, a traditional indicator of nutrient loading. This is an important consideration since much of the current regulatory policy on acceptable nutrient concentrations in surface waters is based on relationships between nutrient concentrations and chlorophyll *a* levels (Dodds and Welch 2000). In this study, chlorophyll *a* was a poor indicator of the strong nutrient enrichment effects on detrital resources and overall ecosystem function in this stream. Thus, when assessing the impacts of nutrient enrichment on streams, the relative importance of both autotrophic and detrital resources should be considered.

References

Bernhardt, E. S. and G. E. Likens. 2004. Controls on periphyton biomass in heterotrophic streams. Freshwater Biology 49: 14-27.

Chadwick, M. A. and A. D. Huryn. 2003. Effect of a whole-catchment N addition on stream detritus processing. Journal of the North American Benthological Society 22: 194-206.

Cross, W. F. 2004. Nutrient enrichment of a detritus-based stream ecosystem: effects on invertebrate community structure and function. Dissertation. University of Georgia Athens, GA, USA.

Dodds, W. K. and E. B. Welch. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society 19: 186-196.

Elwood, J. W., J. D. Newbold and A. F. Trimble. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146-158.

Grattan II, R. M. and K. Suberkropp. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. Journal of the North American Benthological Society 20: 33-43.

Gregory, S. V. 1980. Effects of Light, Nutrients, and Grazing on Periphyton Communities in Streams. Oregon State University

Gulis, V., A. D. Rosemond, K. Suberkropp, H. S. Weyers and J. P. Benstead. 2004. Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. in press, Freshwater Biology.

Gulis, V. and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48: 123-134.

Hill, W. R. and A. W. Knight. 1988. Nutrient and light limitation of algae in two northern California streams. Journal of Phycology 24: 125-132.

Hill, W. R., P. J. Mulholland and E. R. Marzolf. 2001. Stream ecosystem responses to forest leaf emergence in spring. Ecology 82: 2306-2319.

Howarth, R. W. and S. G. Fisher. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. Freshwater Biology 6: 221-228.

Kaushik, N. K. and H. B. N. Hynes. 1971. The fate of dead leaves that fall into streams. Archiv für Hydrobiologie 68: 465-515.

Lowe, R. L., S. W. Golladay and J. R. Webster. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. Journal of the North American Benthological Society 5: 221-229.

Meyer, J. L. and C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. Freshwater Biology 13: 177-183.

Mosisch, T., D., S. E. Bunn and P. M. Davies. 2001. The relative impoartance of shading and nutrients on algal production in subtropical streams. Freshwater Biology 46: 1269-1278.

Ramírez, A. 2001. Control of benthic assemblages in detritus-based tropical streams. Ph.D. Dissertation. University of Georgia Athens, Georgia, USA.

Robinson, C. T. and M. O. Gessner. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia 122: 258-263.

Rosemond, A. D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia 94: 585-594.

Rosemond, A. D., P. J. Mulholland and S. H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Canadian Journal of Fisheries and Aquatic Sciences 57: 66-75.

Tank, J. L. and W. K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48: 1031-1049.

Triska, F. J., V. C. Kennedy and R. J. Avanzino 1983. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. Dynamics of Lotic Ecosystems. T. D. I. Fontaine and S. M. Bartell. Ann Arbor, Ann Arbor Science Publishers: 129-159.

Wellnitz, T. A., R. B. Rader and J. V. Ward. 1996. Importance of light and nutrients in structuring an algal community in a rocky mountain stream. Journal of Freshwater Ecology 11: 399-413.

Winterbourn, M. J. 1990. Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. Freshwater Biology 23: 463-474.