DETERMINATION OF LIMITING NUTRIENT FOR PERIPHYTON GROWTH IN PIEDMONT STREAMS

by

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(Under the Direction of Miguel Cabrera)

ABSTRACT

Nonpoint-source pollution from agricultural and urban sources is a major cause of N and P over-enrichment of aquatic ecosystems. Nutrient enrichment can lead to anthropogenic eutrophication of surface waters, decreasing their ecological, economic, and recreational value. Attached algal communities (periphyton) can be an indicator of stream trophic status. Limitation of periphyton growth by N and P or both was determined using both nutrient-diffusing bottles and nutrient-diffusing agar vials at eight stream sites in two Georgia Piedmont watersheds. Using the nutrient-diffusing bottles, only one site out of seven was limited by N in spring 2005, and no sites were limited by P. The NO_3-N concentration in the N-limited stream was 2.9 mg L^{-1}. In contrast, no N limitation was found and P-limitation was indicated for four sites out of seven in spring 2005 when using nutrient-diffusing agar vials. Stream concentrations at P-limited sites were below 0.03 mg PO_4-P L^{-1}.

INDEX WORDS: passive nutrient diffusing substrate, nutrient enrichment, periphyton, nutrient limitation, Piedmont streams

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DEDICATION

For Darrell, whose patience and support has been unwavering, my parents who have always encouraged me to pursue my goals no matter how difficult or out of the ordinary they may seem, and my grandfathers, who valued service to others and education.

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INTRODUCTION

Nonpoint pollution from agricultural and urban sources is a major cause of N and P overenrichment of aquatic ecosystems. This overenrichment leads to anthropogenic eutrophication, an increasing problem in the United States (Carpenter et al., 1998). To meet nutrient criteria in Ecoregion IX, which encompasses the southeastern temperate forested plains and hills of the United States, waters should not exceed 0.037 mg P L^{-1} and 0.69 mg N L^{-1} (USEPA, 2000). Because of differences in land use and the natural heterogeneity of elemental concentrations in surface water inputs, these N and P threshold levels may not apply to all Ecoregion IX streams. Furthermore, the theory that P is limiting in freshwater and N is limiting in marine waters does not hold for all streams (Gibeau and Miller, 1989; Matlock et al., 1999). The first step in determining appropriate nutrient levels is to establish nutrient limitation. Periphyton growth is a good indicator of stream trophic status. Stream periphyton includes microscopic autotrophs living attached to surfaces and usually associated with heterotrophic microbes and an extracellular matrix of organic matter (Allan, 1995).

The objective of this study was to determine if N, P, or both are the limiting nutrients for periphyton growth in streams in two Georgia Piedmont watersheds, Greenbrier Creek and Rose Creek, located in Greene and Oconee Counties. Land usage in these watersheds is characterized as agricultural and forested. Two types of nutrient-diffusing substrates were employed as artificial surfaces for algal growth. One consisted of bottles diffusing solutions of N, P, N+P, or a deionized water control through a Durapore membrane and glass fiber filter. The other had vials containing N, P, N+P nutrient enriched agar, or a control agar with no nutrient addition, with

nutrients diffusing through a porcelain disk. The bottles and vials were attached to a floating PVC frame, known as a periphytometer (Matlock et al., 1998), and submerged in the streams for 21 to 23 d. The algal biomass on the filters and the porcelain disks were determined by spectrophotometric analysis of chlorophyll *a* (Biggs and Kilroy, 2000).

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CHAPTER 1

LITERATURE REVIEW

Factors Affecting Algal Biomass

A limiting factor hinders the growth or continued growth of an organism or community of organisms when not present in sufficient amounts. Both biotic and abiotic factors limit periphyton growth in the lotic ecosystem. Nutrients limit periphyton-associated algal growth in streams when one or more of the nutrients necessary to increase the algal population is not present in sufficient concentrations. For example, in laboratory studies of systems with high bacterial populations in the presence of sufficient carbon (C), the uptake of nutrients by the large bacterial population decreases nutrient availability and limits algal growth (Cole, 1982). The nutrients necessary for algal population growth are sequestered by the bacterial population and the resulting low nutrient concentrations are the limiting factors in algal growth. When all necessary nutrients are present, increased algal growth can occur and may reach harmful levels because of other abiotic factors.

Algal growth is affected by several biotic and abiotic factors. Biotic factors are competition, parasitism, symbiosis, amenalism, commensalism, and grazing. Competition may or may not affect algal growth. Cole (1982), as discussed in the previous paragraph, gives examples that competition between algae and bacteria in laboratory studies does occur. Whereas, Rier and Stevenson (2002), in a stream in north central Kentucky, found no evidence that competition

with bacteria for inorganic nutrients negatively affected algal growth. They observed that both algal and bacterial growth was positively affected by light. Bacterial growth responded to the addition of glucose and inorganic nutrients with a 70% increase in bacterial biomass, whereas under the same conditions, the increase in algal biomass was 16% and not statistically significant. The mean stream temperature for this experiment was low, at 5°C, and may have hindered any algal growth. The optimum temperature for algal growth, in general, is between 20°C and 28°C.

With regard to invertebrate grazing, there are marked differences in stream systems with and without grazers. When snails, the primary grazer, were removed from a central Tennessee stream habitat, periphyton biomass and productivity increased during all seasons (Rosemond et al., 2000). Algal biovolume in the presence of nutrient and light additions increased dramatically when the snails were removed, from 0.03×10^6 μm^3 mm^{-2} to 25×10^6 μm^3 mm^{-2}. A study on periphyton grazing by caddis fly larvae in a northern California stream proved a decrease in periphyton biomass was inversely proportional to the caddis fly biomass (Hill and Knight, 1988). Periphyton ash-free dry mass (AFDM), a measure of biomass, averaged 5 g m^{-2} with no caddis fly present and 1 g m^{-2} when caddis fly AFDM averaged 40 g m^{-2}. Winterbourn (1990) included the insecticide malathion in a study of periphyton response to nutrient additions in a New Zealand mountain stream to test the impact of grazers on the periphyton growth. Insect densities were only affected at one of the two sites and no significant difference in periphyton biomass was found.

Abiotic factors that affect algal growth are temperature, radiation, dissolved oxygen (DO), and season. In a central Tennessee stream, irradiance and nutrient level were found to be limiting throughout the year, but their importance for algal growth shifted with seasons

(Rosemond et al., 2000). Nutrient diffusion of substrates containing 210 μg NO_3-N L^{-1}, 40 μg NH_4-N L^{-1}, and 35 μg PO_4-P L^{-1} had greater effects on biomass in fall and spring when irradiation was low. In summer, when irradiation was higher, algal growth was not as limited by nutrients. During the spring and fall, in streams not limited by grazers, the addition of light to streams amended with nutrients produced a 100% increase in periphyton biomass. Seasonal nutrient additions to a New Hampshire mountain stream, in the form of nutrient-diffusing substrates containing 7 g NO_3-N L^{-1} and 15.5 g PO_4-P L^{-1}, did not affect periphyton biomass in the summer and only inhibitory effects of N were measured in the fall and spring (Bernhardt and Likens, 2004). Mosisch et al. (2001) studied shading and nutrient effects on Australian streams. They calculated percentage canopy cover by riparian vegetation to compare the performance of nutrient-diffusing substrata in open and forested streams. Shading was determined to be the overall limiting factor controlling algal biomass. For all nutrient treatments, the nutrient substrata at sites with less than 57% canopy cover had between 2.5 and 4 times the amount of periphyton biomass as sites with greater canopy cover.

DO is considered an important parameter in measuring stream water quality. DO in stream water is affected by surface turbulence, temperature, photosynthesis, and respiration. Typically, DO concentrations increase during the day in response to algal photosynthetic activity (Ice and Sugden, 2003) and decrease at night when photosynthetic organisms use oxygen in respiration. Heiskary and Markus (2001) observed the relationships between algae, nutrients, and biochemical oxygen demand (BOD) in five Minnesota rivers. BOD is a measure of how much dissolved oxygen is consumed as microorganisms break down organic matter. A large BOD means that levels of DO will be low. They found that rivers with large nutrient concentrations had greater BOD than rivers with small nutrient concentrations, which had decreased algal

growth. In one river, the BOD high and low average values (11 mg O_2 L^{-1} and 2 mg O_2 L^{-1}) occurred at the same times as the high (160 µg chl *a* L^{-1}) and low (30 µg chl *a* L^{-1}) average values for chlorophyll *a*, a measure of algal biomass. Eutrophication models with algae, aquatic insects, and fish as factors illustrate the impact of trophic level on DO and show that the size of the algal population directly affects the DO level of the system (Yang and Sykes, 1998). When algal population peaks occurred so did elevations in DO level. An increase in algal population by 25 times resulted in a 30% increase in DO.

Point Source Manipulations of Nutrient Concentration

Nutrient limitation in freshwater streams is still not well understood. Nutrient enrichment studies of stream waters have taken several forms, including both point source and nonpoint source additions. The use of nutrient-diffusing surfaces represents point source nutrient enrichment. Fairchild and Lowe (1984) filled clay flowerpots with nutrient-enriched agar with 3.1 g PO_4-P L^{-1} and 1.4 g NO_3-N L^{-1}, sealed them with plastic Petri dishes, and submerged them in a Michigan lake where the nutrients slowly diffused through the clay. The treatments were submerged for up to 36 days. When retrieved, the water surrounding each pot was collected by capping the pot with a beaker before removing it from its location in the lake. The periphyton was scraped off the walls of the clay pot and added to the water sample, which was then analyzed for chl *a* by fluorometry. Algal biovolume was increased ten-fold with increased P, but not with increased NO_3^-, suggesting that the lake was P-limited. Later, using the same technique, they determined that nutrient limitation may be algal species specific (Fairchild et al., 1985). In another study, Tank and Dodds (2003) used 60-mL plastic containers filled with nutrient-

amended agar and covered with either glass fiber filter or a 1 mm-thick, untreated white oak disk to provide inorganic and organic surfaces for periphyton growth in ten North American streams. They used concentrations of 7 g NO_3-N L^{-1} or 15.5 g PO_4-P L^{-1}. After 19 to 23 days, the filters and disks were analyzed for chl *a* either by spectrophotometry or fluorometry. They also collected algae from the epilithon. The type of surface used, inorganic or organic had no effect. Nutrient limitation was examined and found in approximately half of the ten streams tested. For the treatments that used glass fiber filters, there were significant positive responses to N enrichment in 3 streams, no significant positive response to P, and significant positive responses to N+P interaction for 3 streams. The chl values varied by location, but averaged between 0.5 and 10 μg chl *a* cm^{-2}. The treatments that had the white oak disk as the diffusing substrate, showed significant positive responses to N enrichment for 5 streams, no significant positive response to P enrichment, and significant positive responses to N+P enrichment for 1 stream. The amount of chl *a* colonizing the white oak disk was less than on the glass fiber filters, which averaged between 0.1 and 2.5 μg chl *a* cm^{-2}, except in two sites located in Kansas and Arizona in which several treatments yielded between 15 and 20 μg chl *a* cm^{-2}.

Flow-through enclosures allow for increased control of environmental variables and represent another type of point-source nutrient manipulation. The enclosure can either divert water streamside or be placed within the stream. Nutrients are added to the water at the head of the enclosure and changes in the environment within the enclosure can be observed and controlled. Streamside channels were used to test N and P limitation and snail-grazing effects on periphyton biomass in a Tennessee stream. There were three treatments. An N treatment consisting of 210 μg NO_3-N L^{-1} and 40 μg NH_4-N L^{-1}, a P treatment of 35 μg K_2HPO_4 L^{-1}, and an N+P treatment of both the N and P treatments combined. Biomass increased most when N and

P were added together although significance is not stated. The peak chl *a* values when periphyton was not limited by grazing were 25 μg L^{-1} for the N+P treatment, 10 μg L^{-1} for the P treatment, 7 μg L^{-1} for the N treatment, and 5 μg L^{-1} for the control (Rosemond et al., 1993). Nutrient limitation in a tundra stream was tested by creating flow-through enclosures within the stream and altering the nutrient concentration within the enclosure. The concentrations of nutrients in the water within the enclosures were elevated to 100 μg NO_3- N L^{-1} and 10 μg PO_4-P L^{-1} for N,P, and N+P treatments. Microscopic slides provided a surface for periphyton growth. After 2 to 6 days, the slides were removed and assayed for chl content and CO_2 uptake. Nitrogen treatments did not increase periphyton growth, but both P and N+P treatments did. The chl *a* increased from approximately 2.98 μg per slide for the control to between 29.8 and 44.7 μg per slide for the P and N+P treatments (Peterson et al., 1983). In a later study, the biological response to whole-system fertilization of a tundra river was investigated. Algal growth increased in the first 2 years after P additions (Peterson et al., 1993). The P level in the river was increased by 10 μg L^{-1} using a continuous drip and the chl *a* levels were measured on riffle rocks and porcelain tiles. In the first two years of P additions, the chl *a* exceeded 20 μg L^{-1} but then fell to below 3 μg L^{-1} in the third year.

McCormick et al. (2001) used mescosms to carry out a P-limitation experiment for the Florida Everglades. Each mesocosm consisted of a clear plastic cylinder (1.2 m high and 1.5 m in diameter) pushed and anchored 10 cm into the sediment. Holes (3 cm in diameter) capable of being opened and closed were constructed on the sides so that water could be circulated through the mesocosm. Once a week, the mesocosm was closed and P was added at one of six different rates: 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 g P m^{-2}. After 24 h, the mesocosm would be opened again so that water could circulate through. At 5 months, the oligotrophic algal assemblage was

replaced by eutrophic floating mats consisting of cyanobacteria and diatoms at high P loads and by diffuse filamentous chlorophyte mats at intermediate loads. Also, the gross primary productivity rates increased in proportion to the P loading rate. Mesocosms exposed to lower P loads (0.4 to 0.8 g P m^{-2}) did not differ significantly from control mesocosms, whereas mesocosms with high P loading rates (1.6 to 12.8 g P m^{-2}) experienced increased primary productivity due, at first, to increased metabolism in the pre-existing algal assemblage, and later, to dramatic changes in the taxonomic composition of the algae. The gross primary productivity in control mesocosms ranged between 1 and 3 mg O_2 (g AFDM)$^{-1}$ per unit light and for the mesocosms receiving high P loads it averaged between 2 and 7 mg O_2 (g AFDM)$^{-1}$ per unit light. This change in algal assemblage from oligotrophic to eutrophic form affected the nutrient storage capacity of the wetland as a whole. Over time, the water P concentration increased in the water at further distances from the source of P input.

Limiting nutrient determination for periphyton growth in an Oklahoma woodland stream was tested by Matlock et al. (1998) with the use of periphytometers, which are floating or anchored racks that hold nutrient diffusing bottles in replicate just under the surface of the water. The nutrients in solution diffused out of a hole cut in the cap of each bottle covered by a dialysis membrane and glass fiber filter. The dialysis membrane controlled the rate of diffusion and also acted as a biofilter to prevent contamination of the solution in the bottle by algae or bacteria while the glass fiber filter served as a textured surface ideal for periphyton growth. Four nutrient solutions were used: N as 36 mg NO_3-N L^{-1}, P as 20 mg PO_4-P L^{-1}, N+P combined at the same concentrations, and a deionized water control. Ten replicates of each solution were placed in the bottles and submerged in the stream for up to 2 weeks. After deployment, the glass fiber filters were extracted for chl *a*, which was then quantified by fluorometry, and used as a measure of

algal biomass. They determined that the stream was P limited. The P treatments yielded an average of 3.44 μg chl *a* cm^2, with 1.29 μg chl *a* cm^2 in response to the N treatment, and 1.46 μg chl *a* cm^2 in response to the control. Matlock et al. (1999a, 1999b) then used the periphytometer technique to develop a method for determining stream ecosystem trophic status by comparing the baseline periphyton primary production to the maximum potential primary production. Baseline production describes growth in the absence of nutrient addition, while maximum potential production occurs with nutrient enrichment.

The periphytometer has been employed by Carey (2005) for nutrient limitation studies of nine coastal plain rivers in southern Georgia. Treatments used were 87.5 mg NO_3-N L^{-1}, 12 mg PO_4-P L^{-1}, N+P at the same concentrations, and a deionized water control. Chl *a* was quantified by fluorometry. Periphytometer deployments in shaded sites did not measure periphyton growth response to nutrient enrichment. All but three periphytometer deployments in unshaded, high irradiance sites had at least one treatment produce significantly higher chl *a* values when compared to the control. Average chl concentrations ranged between 0.2 and 3.5 μg cm^2 for the control treatments and between 1 and 7 μg cm^2 for the N, P, or N+P nutrient treatments showing positive periphyton growth response.

Gibeau and Miller (1989) tested for nutrient limitation using nutrient-enriched agar vials containing 390 mg PO_4-P L^{-1} or 13 mg NH_4-N L^{-1}. They covered the openings with porcelain disks and submerged them in an Alaskan river for 3 weeks. Upon retrieval, the disks were removed and analyzed for chl *a* by fluorometry. They determined the river was limited by N+P. Average chl biomass for the N+P treatment was 1.23 μg cm^{-2} chl *a*, for the P treatment was 0.56 μg cm^{-2} chl *a*, for the N treatment was 0.26 μg cm^{-2} chl *a*, and for the control was 0.33 μg cm^{-2}

chl *a*. The N+P treatment produced significantly more chl *a* than the P, N, and control treatments, which were not significantly different from each other.

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CHAPTER 2

PRELIMINARY TESTING OF NUTRIENT-DIFFUSING SUBSTRATES

Introduction

Nutrient limitation in surface waters has been tested using many different methods, including whole lake or stream fertilization (Schindler, 1974; Peterson et al., 1993), flumes placed in-stream or that divert stream water streamside (Rosemond et al., 1993), nutrient agar-filled clay pots (Fairchild and Lowe,1984), bottles diffusing nutrients through a glass-fiber filter substrate for periphyton growth (Matlock et al., 1998), and agar-filled vials diffusing nutrients through a wooden or porcelain substrate (Gibeau and Miller, 1989).

Matlock et al. (1998) developed the periphytometer, a floating or streambed anchored rack to carry nutrient diffusing bottles in replicate, to test streams for periphyton growth limitation by N, P, or N+P in streams in Oklahoma. Tests on diffusion rate were performed for various concentrations and amounts of solution used in the field experiments. One-liter bottles with 2.5-cm diameter holes cut in the lids, a 0.45-μm cellulose semi-permeable dialysis membrane over the mouth of the bottle, and a glass fiber filter over the dialysis membrane were used. The initial concentrations of the nitrate and phosphate solutions placed in the bottles were 8.1 mM $NaNO_3$ and 1.5 mM $Na_2HPO_4 \cdot 7H_2O$. The bottles were submerged and placed on their sides with lid surface perpendicular to the surface of the water in a flume with flow at 1 m s^{-1}. The water was from a local reservoir with relatively low nutrient concentrations. Over a 27-d

period the conductivity of randomly chosen bottle solutions and the ambient water was measured 8 times. The observed diffusion rate of the nitrate solution was 27 μg cm^{-2} h^{-1} and for the phosphate solution was 17 μg cm^{-2} h^{-1}. After 27 days in the flume, the nutrient solution initial concentrations had decreased by 25% for nitrate and 22% for phosphate.

Carey (2005) tested the diffusion rates for 20-mL scintillation vials with 1.6-cm diameter holes drilled in the caps. The 0.45-μm dialysis membrane and glass fiber filter were placed over the opening and held in place by the cap. The vials were filled with an N+P solution at an initial concentration of 87.5 mg NO_3-N L^{-1} and 10 or 12 mg PO_4-P L^{-1}. A trough was built with PVC pipe that was cut open on one side and set at an angle so that tap water could run through the trough at 0.05 m s^{-1}, a typical flow for coastal plain rivers. The tap water was relatively low in nutrients. The scintillation vials were placed on their sides in the trough so that they would be held just under the surface of the water and the top of the scintillation vial would be perpendicular to the surface of the water. Two experiments were run. The first experiment used a pipette to take water samples each day close to the diffusing surface of the vial that was to be removed on the final day of the experiment. The second experiment analyzed the nutrient concentration of one filter each day. For both experiments, one scintillation vial was removed each day for 10 and 15 d and analyzed for remaining nutrient concentration. At the end of both experiments, the N concentration in the bottles had decreased by 94.9% in 10 d and by 97.5% in 15 d, whereas P concentration in the bottles had decreased by 74.3% in 10 d and and by 84.2% in 15 d.

The objective of this experiment was: to test the diffusion rate, while in-pseudostream, of nutrient solution-filled bottles with glass fiber filters and agar-filled vials with porcelain disks; and to compare, while in-stream, periphyton growth on bottles with glass fiber filters and on

agar-filled vials with porcelain disks. For purposes of clarity, the bottle/filter system will be referred to as either "bottle" or "filter" and the vial/disk system will be referred to as "vial" or "disk".

Materials and Methods

Nutrient-Diffusing Bottles

For this experiment, 24 60-mL narrow-mouth Nalgene bottles (Nalge Nunc International, Rochester, NY, USA) were used. A 1.5-cm hole was cut in the cap of each bottle and care was taken so the size of the hole in each cap was identical (Fig 2.1). Each bottle was acid-washed and filled with the same concentration of N+P solution: 87 mg NO_3-N L^{-1} + 12 mg PO_4-P L^{-1} (KNO_3 + Na_2HPO_4 in deionized water; Carey, 2005). These concentrations are above the maximums set at 0.037 mg P L^{-1} and 0.69 mg N L^{-1} as ambient nutrient concentration for surface waters in Ecoregion IX, and below toxic levels for algae growth (USEPA, 2000). The 60-mL bottles were filled to capacity with the N+P solution using a repipet, each bottle receiving 63 mL of solution. A 25-mm diameter 934/AH glass-fiber filter (Whatman, Middlesex, UK) was cut to 16 mm using a punch and placed inside the lid, covering the hole and held in place by the underside of the lid. A 25-mm diameter, 0.45-µm Durapore dialysis membrane (Millipore, Billerica, MA, USA) was placed on the opening of the filled 60-mL bottle and the cap with cut glass-fiber filter was carefully placed over the membrane and screwed in place. The dialysis membrane provided some control of diffusion and the glass-fiber filter acted as a substrate for periphyton growth.

The Experimental Creek

The experimental creek was a pseudostream constructed and operated in the laboratory from a PVC pipe that was 20 cm in diameter and 1.8 m in length and set up indoors next to a window. One side of the pipe was cut off so that when laid flat the entire length of the inside of the pipe was visible. It was supported by wooden blocks with one end lowered so that water would flow out of that end. Both ends were capped and a hole was cut in the lowered end. Plastic-coated, wire lawn fencing, forming a grid with 5x7.5-cm squares, was cut to fit the length of the experimental creek and affixed to the pipe with large zip-ties so that when the bottles were attached to the fencing they would be held 7.5 cm apart and under the surface of the water. They were attached with zip-ties to the fencing so that the bottles were oriented sideways and the nutrient-diffusing surface of the cap was perpendicular to the surface of the water. A 167-L container was acid-washed, filled with approximately 110 L of deionized water, and placed under the lowered end of the pipe. A submersible pond pump (Garden Treasures, Lowe's, North Wilkesboro, NC, USA) with a 2.5-cm diameter hose attached to the outflow was placed in the 167-L container. The outflow hose was extended to the elevated end of the experimental creek to circulate water. A bypass with adjustable water volume control was inserted into the hose between the pump and the end that dispensed water into the elevated side of the creek to control the volume of water running through the apparatus. Another hose diverted the extra water from the bypass back into the 167-L container. The flow was adjusted to 0.33 m s^{-1} to mimic flow measured in Georgia Piedmont streams. Water flowing through the experimental creek exited through the hole cut in the cap at the lowered end and fell back into the 167-L container. (Fig 2.2)

On the first day and every 3 d thereafter, 3 bottles were randomly sampled. They were removed from the pseudo stream, the solutions were poured into 20-mL scintillation vials, and then stored at 4°C. At the same time, the background N and P water concentrations were sampled from the 167-L container and stored at 4°C in scintillation vials. Each sampled solution was tested for NO_3-N with the Gries-Ilosvay procedure after reduction of NO_3^- to NO_2^- (Mulvaney, 1996), and for PO_4-P with the molybdate-blue method (Murphy and Riley, 1962). On sampling days, after a sample of the container water was taken, the circulating water in the 167-L container was replaced with fresh deionized water. This kept the background concentration of the experimental creek low.

Temperature Measurements in the Pseudo Stream

A HOBO microstation (Onset Computer Corporation, Bourne, MA, USA) with a sensor for water temperature was used to monitor and record water temperature in the pseudo stream. Measurements were taken in 1-min intervals and logged at 2-min intervals.

Nutrient-Diffusing Agar Vials

The experimental creek design was used to test the diffusion rate of agar-filled vials with porcelain disks for periphyton growth (Gibeau and Miller, 1989). The plastic vials were of 30-mL capacity with snap tops (Poly-cons, Madan Plastics, Inc., Cranford, NJ, USA). A 1.9-cm hole was cut in the top of each vial and a 2.7-cm porcelain disk was affixed to the opening by heating the disk on a hot plate, allowing it to melt to the surface of the cap, completely covering the hole, and then cooling the disk and cap by dropping in a beaker of water (Fig 2.3; William Perry, personal communication).

Ten vials were filled to the top with agar that contained one of five NO_3-N+ PO_4-P concentrations. (Table 2.1) Each agar was prepared by first placing 2 g of powdered agar, granulated solidifying agent (Fisher Scientific), into a 500-mL glass bottle. Then the appropriate amount of KNO_3 and $Na_2H_2PO_4$ was added to a graduated cylinder and brought up to 500 mL volume with deionized water. When the solids were completely dissolved, usually with agitation and heat, the solution was added to the glass bottle with the dry agar. The bottle was placed on a hot plate with a stir bar and heated and stirred until the liquid agar was ready to be poured into the vials. Upon cooling, the tops with fused porcelain disks were snapped into place.

The same experimental creek design used for the bottles was employed for the vials. Two PVC experimental creeks were constructed and placed outside in the sunlight. The same water supply from a single 167-L container and submergible pump was used for both experimental creeks. Instead of deionized water, tap water was used. The vials were placed upright with the surface of the porcelain disk parallel to and just below the surface of the water. They were attached to the plastic-coated, wire lawn fencing with zip-ties and exposed for 5 d. Vials were sampled at time 0 and again at day 5 and stored at 20°C until extraction.

At the time of analysis, the agars were removed from the vials, ground for 3 seconds each using a hand mixer, and placed in a 1-L bottle with deionized water. The 87 mg NO_3-N L^{-1} + 12 mg PO_4-P L^{-1} agars were extracted with 400 mL of deionized water at time 0 and with 200 mL of deionized water at day 5. All other agar concentrations were extracted with 800 mL of deionized water. Each bottle was placed on a shaker at 120 oscillations min^{-1} for 2 h. Then the extract solutions were diluted and tested PO_4-P with the molybdate blue method (Murphy and Riley, 1962). Three agars of each concentration sampled at 5 d were sliced into four 0.9-cm thick layers and numbered 1 to 4. Layer 1 was the top layer of the agar in the vial and layer 4 was the bottom

layer. Each slice was extracted with deionized water and analyzed for PO_4-P as described above. The NO_3-N remaining in the agars was not determined due to time constraints.

In-Stream Deployment of Bottle/Filter and Vial/Disk Systems

Filters and disks were prepared as described earlier in the Methods section. Treatments included a control (no nutrient added), N, P, and N+P. Concentrations for N treatments were 78 mg NO_3-N L^{-1} for bottles and 7,000 mg NO_3-N L^{-1} for vials. Concentrations for P treatments were 12 mg PO_4-P L^{-1} and 15,500 mg PO_4-P L^{-1} for vials. Ten replicates of each treatment were randomly placed on two periphytometers, one with bottles and one with vials. The bottles were attached with the filter face perpendicular to the water surface, whereas the vials were attached with the porcelain disk face parallel to the water surface. A periphytometer with bottles and a periphytometer with vials were deployed at eight sites on two streams the spring and summer of 2005. Each periphytometer consisted of a frame constructed with 3-cm diameter PVC pipe. Plastic-coated wire fencing, forming a grid with 5 x 7.5-cm squares, was cut to fit the width and length of the frame and attached to the side that would float just under the surface of the water (Fig 2.4). Periphytometers were exposed to stream flow for 21 to 24 d and then the filters and disks were analyzed for µg chl *a* cm^{-2}.

Site Description

Eight sites were used for this study. Four on each of the Greenbrier and Rose Creeks located in the Georgia Piedmont in Oconee and Greene counties. On Rose Creek, two sites are adjacent to hay fields (G, H), one is forested (F), and one is deforested, planted with grass, and the channel altered with sand and rocks just downstream from a road crossing (E). On the

Greenbrier Creek, one site is adjacent to pasture (B) and three are forested (A, C, D). The cattle from the pasture adjacent to Greenbrier Creek were not allowed in the channel upstream from the study site. The study sites are located at the end of a run and just downstream from a stormflow collector and baseflow sampling sites. All sites have at least 7.5 m of riparian zone, though canopy cover allows direct sunlight at midday with the exception of site A.

Abiotic Measurements in Stream

At each stream site, a HOBO microstation (Onset Computer Corporation, Bourne, MA, USA) was used to measure and record photosynthetically active radiation (PAR) and water temperature. Measurements were collected every minute for the entire duration of each deployment. Stream gradient at each site was detemined with a clinometer.

Statistical Analyses

Generalized linear model analysis (PROC GLM in SAS 9.1) was used to carry out an analysis of variance for the amounts of PO_4-P diffused from agars at five concentrations, as well as for chl *a* concentrations in filters and disks from different treatments deployed in the periphytometers (SAS Institute, Cary, North Carolina). Also, stepwise regression analysis (PROC REG) was used to relate chl *a* concentration on filters and disks to environmental variables such as photosynthetically active radiation (PAR), temperature, stream NO_3-N and stream PO_4-P concentrations, and stream gradient.

Results and Discussion

Nutrient-Diffusing Bottles

The NO_3-N and PO_4-P concentrations in the bottle solution decayed exponentially, more rapidly at first and then slowing near the end of the experiment (Table 2.2, Figure 2.1). The solution concentrations decreased by almost 41% NO_3-N and 27% PO_4-P in the first 3 d, and by 63% NO_3-N and 41% PO_4-P by the sixth day. At the midpoint, 12 d, of the experiment, the bottle nutrient concentrations had decreased by 84% NO_3-N and 63% PO_4-P. At removal, 24 d, the N solution had decreased from 78.81 mg NO_3-N L^{-1} to 1.77 mg NO_3-N L^{-1}, a decrease of 98%. The P solution had decreased from 12.55 mg PO_4-P L^{-1} to 1.68 mg PO_4-P L^{-1}, a decrease of 87%. For both, NO_3-N and PO_4-P, the final concentrations were above the nutrient criteria for ecoregion IX (0.036 mg P L^{-1} and 0.69 mg N L^{-1}), suggesting that for up to 24 d these nutrient-diffusing bottles could expose periphyton growing on the glass-fiber filters to concentrations that would be sufficient to stimulate algal growth in responsive sites.

The average temperature of the circulating water during the experiment was 25.6 °C, and the PO_4-P concentration in the circulating water stayed below the median value of 0.017 mg P L^{-1} measured by Franklin et al. (2002) in Piedmont streams (Table 2.3) The NO_3-N concentration was rather elevated during the first week, reaching a peak of 0.33 mg NO_3-N L^{-1}, and then decreasing to very low levels. This increase in NO_3-N concentration in the circulating water was likely caused by the rapid initial loss of NO_3-N from the bottles. Because the circulating water was changed every 3 d and the loss of NO_3-N slowed with time, the circulating water NO_3-N concentration rose only in the first 9 d of the experiment and then remained low.

The NO_3-N and PO_4-P concentrations measured in the bottles are comparable to those obtained by Carey (2005) when using 20-mL vials over a 15-d exposure period. At 15 d, the NO_3-N loss from the bottles in this experiment was 76% PO_4-P and 93% NO_3-N, which matches closely with 74% PO_4-P and 95% NO_3-N found by Carey (2005). These similar results were obtained despite the difference in water velocities used. Carey used 0.05 m s^{-1} to mimic a coastal plain river and this experiment used 0.33 m s^{-1} to mimic a Piedmont stream. The nutrient loss from the bottles tested by Matlock et al. (1998) is not consistent with the nutrient loss determined for this experiment. Matlock et al. found that after 27 d in a flume with water velocity of 1 m s^{-1}, the NO_3-N and PO_4-P concentrations had decreased by only 25% and 22%, respectively.

Nutrient-Diffusing Agar Vials

The average water temperature in the experimental creeks during the agar vial exposure period was 24.4 °C. Only the PO_4-P concentrations of the agar vials were tested. At 5 d, the agar concentrations decreased between 3% and 15%, but there were no differences between concentrations (Table 2.4). Because we knew that the decay of PO_4-P in the agar vials would be exponential, we expected the greatest rate of diffusion to occur in the first 5 d the vials were exposed to the experimental creek. The highest loss of PO_4-P from any of the vials was 15% after 5 d. This rate of loss would be expected to decrease with time, but even if the diffusion rate remained constant (linear), the agar would be expected to lose 75% in 25 days, and would still supply PO_4-P to periphyton growing on the filters after 25 d of exposure. Results obtained when agar slices were extracted further confirmed that the upper 1 cm of the agar was not completely depleted in 5 d (Table 2.5) and therefore would be expected to be able to supply sufficient PO_4-P

for 25-d exposure times. Based on these results, we decided to use concentrations of 15,500 mg PO_4-P L^{-1} and 7000 mg NO_3-N L^{-1} (0.5 M NO_3-N and PO_4-P) for the stream studies.

In-Stream Deployment of Nutrient-free Agar and Vial Treatments

The concentrations of chl *a* ranged from 0.06 µg chl *a* cm^{-2} to 2.1 µg chl *a* cm^{-2} in filters, and from 0.12 µg chl *a* cm^{-2} to 12.1 µg chl *a* cm^{-2} in disks. Analysis of variance of chl *a* concentration in filters and disks indicated a significant site x deployment time x treatment x media interaction. Whereas in some sites, deployment times, and treatments, there were no differences in chl *a* between filters and disks ($p<0.05$), in most cases (11/15 in control, 10/15 in N-treatment, 11/15 in P-treatment, and 12/15 in N+P treatment) disks had significantly greater values than filters (Tables 2.6 through 2.9).

The greater concentrations of chl *a* in disks may have been due to their orientation on the periphytometer. The filters were perpendicular to the surface of the water but parallel to radiation, whereas the disks were parallel to the surface of the water and therefore perpendicular to radiation. It is possible that the level of PAR received by the filters was so low that there was not enough irradiance for the same periphyton community structure that developed in disks. Furthermore, the disks may have provided a more suitable substrate for periphyton growth because of their structure, greater size (2.5 cm diameter vs. 1.6 cm diameter of the filter), and orientation. In addition, filters were not as stable in the stream environment. Over all treatments, 26% of the filters were lost after 21 d to 24 d in the stream for all deployments, whereas only 2% of the disks were lost under the same conditions.

Regression analysis was used to identify factors (PAR, water temperature, stream background N and P concentrations, and stream gradient) affecting chl *a* concentration in filters

and disks for each treatment (Table 2.10). For filters, stream gradient and PAR were the most significant factors. Chl *a* concentration increased with an increase in stream gradient and decreased with an increase in PAR. This effect of PAR was unexpected because, as described above, the orientation of the filters in the stream would have led to a reduction of PAR. An increase in chl *a* concentration would have been expected with increasing PAR. One could speculate that the orientation of the filter causes it to receive only refractory light through the riparian canopy as opposed to direct light that would reach the disks at midday. These results suggest that the organisms growing on the filters have an inverse relationship to light. Stream gradient, PAR, and stream N were the significant variables for the P-treated filters, in which chl *a* concentration decreased as stream N increased ($R^2 = 0.19$). For disks, stream N also had a negative effect on the chl *a* concentration of the N+P and N treatments. Stream P had a positive effect on the chl *a* concentration of the N treatment and temperature had a negative effect on the chl *a* concentration of the control treatment. It is clear that additional research is needed to determine if different organisms grow on filters set parallel to sunlight as compared to disks set perpendicular to sunlight as was the case in this study.

Conclusions

Nutrient-diffusing bottles exposed to an experimental creek experienced rapid loss of nutrients, but final concentrations of NO_3-N and PO_4-P after 24 d of exposure were above the nutrient criteria for ecoregion IX (0.036 mg P L^{-1} and 0.69 mg N L^{-1}). These results suggest that nutrient-diffusing bottles should provide sufficiently high concentrations to stimulate algal growth in nutrient-deficient streams. A maximum of 15% of the initial PO_4-P in nutrient-

diffusing agar was lost during 5 d of exposure in an experimental creek, and no evidence of complete depletion of the PO_4-P in the upper 1 cm of agar was observed. Based on these results, concentrations of 15,500 mg PO_4-P L^{-1} and 7000 mg NO_3-N L^{-1} were selected for later field studies.

When both filters and disks were tested as a substrate for periphyton growth, disks had greater chl *a* concentrations in most comparisons. This may be a result of the orientation of the disk to irradiance. The disks received more irradiance than the filters, which were oriented perpendicularly to the surface of the water and parallel to irradiance. Therefore, the filters may not have received enough irradiance to support the same periphyton community structure as the disks. This is further supported by regression analysis for filters which indicated an inverse relationship between PAR and chl *a* concentration. In conclusion, disks are better for measuring periphyton growth than filters because they have more surface area for growth, they consistently produced greater chl *a* concentrations, and are more resistant to loss in Georgia Piedmont streams.

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Table 2.1. Summary of agar concentrations in mg L^{-1} and the amounts of $Na_2H_2PO_4$ and KNO_3 added for each concentration.

Agar concentration	PO_4-P	$Na_2H_2PO_4$ added to 1 L of agar	NO_3-N	KNO_3 added to 1 L of agar
	mg L^{-1}	g	mg L^{-1}	g
0.5 M	15,500	29.995	7000	25.275
0.25 M	7750	14.998	3500	12.638
0.1 M	3100	5.999	1400	5.055
0.05 M	1550	2.999	700	2.538
12/87 mg L^{-1}	12	0.0298	87	0.297

Table 2-2. Average NO_3-N and PO_4-P concentrations in bottles sampled every 3 d from experimental creek, percentage of initial NO_3-N and PO_4-P remaining in bottles, and amount of total amount of NO_3-N and PO_4-P diffused.

Day	Final average PO_4-P	Final average NO_3-N	Percentage of initial P	Percentage of initial N	Flux of PO_4-P	Flux of NO_3-N
	------mg L^{-1}-----				------mg cm^{-2}-----	
1	12.55	78.81	100	100	0	0
3	9.20	46.71	73.3	59.3	0.11	1.09
6	7.39	29.46	58.9	37.4	0.17	1.68
9	4.25	12.09	33.9	15.3	0.28	2.27
12	4.66	12.75	37.1	16.2	0.27	2.24
15	3.10	5.58	24.7	7.1	0.32	2.49
18	1.83	2.16	14.6	2.7	0.36	2.60
21	2.79	5.40	22.3	6.9	0.33	2.49
24	1.68	1.77	13.4	2.2	0.37	2.62

Table 2-3. PO_4-P and NO_3-N concentrations from 167-L container during bottle diffusion experiment in experimental creek.

Day	PO_4-P	NO_3-N
	----------mg L^{-1}----------	
1	0.002	0.00
2	0.009	0.20
3*	0.013	0.33
5	0.010	0.28
6*	0.007	0.15
7	0.007	0.12
8	0.007	0.07
9*	0.003	0.00
10	0.002	0.01
11	0.002	0.01
12*	0.001	0.01
14	0.002	0.01
15*	0.00	0.01
16	0.002	0.01
17	0.001	0.01
18*	0.001	0.00
19	0.002	0.00
21*	0.001	0.00
22	0.00	0.00
25*	0.001	0.00

* indicates day in which fresh deionized water was circulated through the experimental creek. Sampling on these days occurred before water was changed.

Table 2-4. Summary of mean P concentrations for agars tested in experimental creek.

Initial agar PO_4-P concentration	Concentration at 0 d	Concentration at 5 d	PO_4-P loss	Flux of PO_4-P	% PO_4-P lost
mg L^{-1}	---------- mg----------			mg cm^{-2}	
15,500	443.74	404.08	39.66	6.93 a†	8.94 a†
7750	219.63	212.87	6.76	1.18 a	3.08 a
3100	83.27	70.43	12.83	2.24 a	15.41 a
1550	43.98	40.40	3.58	0.63 a	8.14 a
12	0.79	0.68	0.11	0.02 a	14.48 a

†Within each column, value followed by the same letter are not significantly different according to Fisher's LSD at $p<0.05$.

Table 2-5. Average phosphorus concentration of agar layers after 5 d in experimental creek, with estimated loss and percentage of total agar concentration.

Agar Concentration	Agar depth from surface	Final PO_4-P†	Estimated P loss	Percentage of PO_4-P in whole agar‡
mg L^{-1}	cm	----------mg----------		
15,500	0-0.9	91.37 a	24.93	24.0 a
	0.9-1.8	95.14 a	21.16	25.0 a
	1.8-2.7	94.73 a	21.57	24.9 a
	2.7-3.6	99.28 a	17.02	26.1 a
7750	0-0.9	51.73 a	6.37	26.2 a
	0.9-1.8	51.85 a	6.25	26.3 a
	1.8-2.7	42.31 a	15.79	21.4 b
	2.7-3.6	51.40 a	6.7	26.1 a
3100	0-0.9	17.04 a	6.26	24.8 a
	0.9-1.8	16.60 a	6.7	24.2 a
	1.8-2.7	18.33 a	4.97	26.7 a
	2.7-3.6	16.68 a	6.62	24.3 a
1550	0-0.9	9.85 a	1.75	26.5 a
	0.9-1.8	8.86 a	2.74	23.8 a
	1.8-2.7	9.36 a	2.24	25.2 a
	2.7-3.6	9.14 a	2.46	24.6 a
12	0-0.9	0.026 a	0.064	22.8 a
	0.9-1.8	0.032 a	0.058	27.5 a
	1.8-2.7	0.027 a	0.064	23.1 a
	2.7-3.6	0.031 a	0.059	26.7 a

† initial agar concentration for slices are as follows:
15,500 mg L^{-1}: 116.3 mg
7750 mg L^{-1}: 58.1 mg

3310 mg L^{-1}: 23.3 mg
1550 mg L^{-1}: 11.6 mg
12 mg L^{-1}: 0.09 mg
‡ Within each column and agar concentration, percentage followed by the same letter are not significantly different according to Fisher's LSD at $p<0.05$.

Table 2.6. Comparisons of chl *a* concentrations on control treatments of nutrient-diffusing bottles and agar vials at each site during the spring and summer of 2005.

Site	Mean chl *a* in bottles	Mean chl *a* in vials	p < t
	------μg cm^{-2}------		
	spring 2005		
A	0.09	0.12	0.7553
B	1.25	2.58	0.0063
C	0.07	5.88	0.0133
D	0.06	1.95	<0.0001
E	2.04	4.59	0.001
G	0.47	2.76	0.0018
H	1.84	5.07	0.0301
	summer 2005		
A	0.08	1.59	<0.0001
B	2.09	12.06	<0.0001
C	0.58	3.44	0.0032
D	0.16	2.03	0.0031
E	0.77	8.27	0.0659
F	0.13	0.52	0.067
G	1.36	2.84	0.0048
H	1.07	3.29	0.0946

Table 2.7. Comparisons of chl *a* concentrations on N treatments of nutrient-diffusing bottles and agar vials at each site during the spring and summer of 2005.

Site	Mean chl *a* in bottles	Mean chl *a* in vials	p < t
	------µg cm^{-2}------		
	spring 2005		
A	0.02	0.01	0.85
B	3.02	1.47	0.005
C	3.77	0.54	0.0032
D	1.13	0.32	0.11
E	5.22	5.21	0.99
G	4.06	1.71	0.0002
H	1.11	1.34	0.56
	summer 2005		
A	1.30	0.19	<0.0001
B	7.88	1.62	<0.0001
C	6.65	0.07	0.0016
D	1.95	0.12	<0.0001
E	6.03	0.90	0.037
F	0.37	0.15	0.21
G	3.26	0.39	0.05
H	4.88	2.32	0.046

Table 2.8. Comparisons of chl *a* concentrations on P treatments of nutrient-diffusing bottles and agar vials at each site during the spring and summer of 2005.

Site	Mean chl *a* in bottles	Mean chl *a* in vials	p < t
	------μg cm^{-2}------		
	spring 2005		
A	0.11	0.11	0.98
B	5.04	1.33	0.0008
C	3.13	0.60	0.0008
D	4.48	0.29	<0.0001
E	7.62	1.88	0.0004
G	6.07	1.69	<0.0001
H	4.09	1.47	0.13
	summer 2005		
A	1.56	0.12	<0.0001
B	11.58	1.86	<0.0001
C	8.46	0.08	0.0016
D	3.17	0.09	<0.0001
E	6.44	1.43	0.09
F	0.52	0.08	0.06
G	4.88	0.53	0.016
H	6.08	2.31	0.0037

Table 2.9. Comparisons of chl *a* concentrations on N+P treatments of nutrient-diffusing bottles and agar vials at each site during the spring and summer of 2005.

Site	Mean chl *a* in bottles	Mean chl *a* in vials	p < t
	------µg cm^{-2}------		
	spring 2005		
A	0.09	0.08	0.70
B	4.39	1.78	0.006
C	2.97	0.72	<0.0001
D	2.90	0.17	0.005
E	9.71	7.58	0.30
G	6.22	1.49	<0.0001
H	1.80	1.64	0.66
	summer 2005		
A	1.26	0.19	<0.0001
B	11.72	2.10	<0.0001
C	10.75	0.04	<0.0001
D	2.52	0.07	0.003
E	5.86	1.08	0.05
F	0.95	0.13	0.004
G	4.14	0.35	0.0004
H	3.64	2.07	0.037

Table 2.10. Regression analysis of factors affecting chl *a* concentrations on each treatment for disks and filters.

Media	Treatment	Intercept	Stream gradient (v. m (h. m^{-1}))†	Temperature (˚C)	Stream P (mg L^{-1})	Stream N (mg L^{-1})	PAR (mole photons m^{-2})	R^2
disk	control	21.53		-0.94				0.28
	N	0.01			58.76			0.44
	N+P	0.53	136.75			-1.28		0.44
	P	5.97				-3.41		0.24
filter	control	-0.83	95.82				-0.0067	0.11
	N	-0.86	108.91				-0.0084	0.09
	N+P	-1.58	171.48				-0.0125	0.12
	P	0.97	58.26			-0.77	-0.0101	0.19

† v. m (h. m^{-1}) = vertical meters / horizontal meters

Figure 2.1. A 60-mL bottle with 1.5-cm hole cut in the lid. Dialysis membrane and glass fiber filter are held in place between the mouth of the bottle and the lid.

Figure 2.2. Experimental Creek. Trough cut from 20-cm diameter PVC pipe. 60-mL bottles filled with N+P solution with glass fiber filters are oriented sideways 7.5 cm apart just under the surface of the water. Water is circulated by submersible pump in the 167-L container (at the bottom of the picture) with 2.5-cm diameter hose extended to elevated end of the trough (at the top of the picture). Inset: Nutrient-diffusing bottles held under the surface of the water.

Figure 2.3. Agar vials attached to the periphytometer. The vials are oriented so the porcelain disk is parallel to the surface of the water.

Figure 2.4. Periphytometers with bottles (on the left) and vial (right) attached.

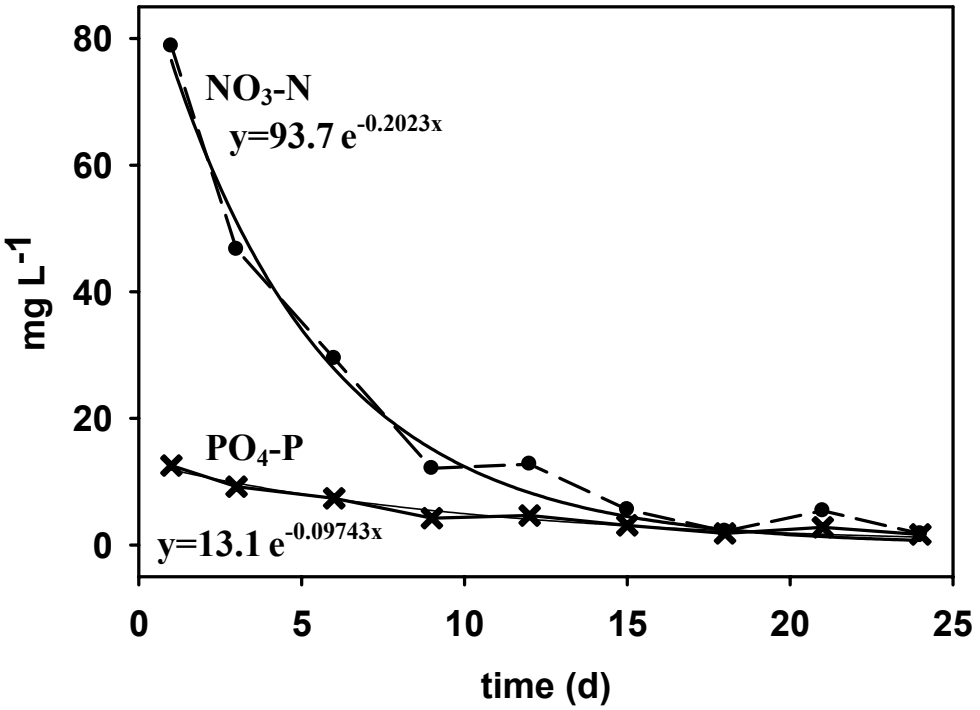

Figure 2.5. NO_3-N and PO_4-P concentrations of bottles sampled from experimental creek every three days. Equations for exponential decay included.

CHAPTER 3

DETERMINATION OF LIMITING NUTRIENT FOR PERIPHYTON GROWTH IN PIEDMONT STREAMS[1]

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[1]C.R. Richards, D.H. Franklin, M.L. Cabrera, D. E. Radcliffe, and M. Risse. To be submitted to *Journal of Environmental Quality*.

Abstract

Nonpoint-source pollution from agricultural and urban sources is a major cause of N and P over-enrichment of aquatic ecosystems. Nutrient enrichment can lead to anthropogenic eutrophication of surface waters, decreasing their ecological, economic, and recreational value. Attached algal communities (periphyton) can be an indicator of stream trophic status. Limitation of periphyton growth by N and P or both was determined using both nutrient-diffusing bottles and nutrient-diffusing agar vials at eight stream sites in two Georgia Piedmont watersheds. Using the nutrient-diffusing bottles, only one site out of seven was limited by N in spring 2005, and no sites were limited by P. The NO_3-N concentration in the N-limited stream was 2.9 mg L^{-1}. In contrast, no N limitation was found and P-limitation was indicated for four sites out of seven in spring 2005 when using nutrient-diffusing agar vials. Stream concentrations at P-limited sites were below 0.03 mg PO_4-P L^{-1}.

Introduction

Nonpoint pollution from agricultural and urban sources is a major cause of N and P over-enrichment of aquatic ecosystems. This over-enrichment leads to anthropogenic eutrophication, an increasing problem in the United States (Carpenter et al., 1998). For nutrient criteria in Ecoregion IX, which encompasses the southeastern temperate forested plains and hills of the United States, waters should not exceed 0.037 μg P L^{-1} and 0.69 mg N L^{-1} (USEPA, 2000). Because of differences in land use and the natural heterogeneity of elemental concentrations in surface water inputs, these N and P levels may not apply for all Ecoregion IX streams.

Furthermore, the theory that P is limiting in freshwater and N is limiting in marine waters does not hold for all streams (Gibeau and Miller, 1989; Matlock et al., 1999a). The first step in determining appropriate nutrient levels is to establish nutrient limitation. Periphyton growth is a good indicator of stream trophic status. Stream periphyton include microscopic autotrophs living attached to surfaces and usually associated with heterotrophic microbes and an extracellular matrix of organic matter (Allan, 1995).

Nutrient limitation in surface waters has been tested using many different methods, including whole lake or stream fertilization (Schindler, 1974; Peterson et al., 1993), flumes placed in-stream or that divert stream water streamside (Rosemond et al., 1993), nutrient agar-filled clay pots (Fairchild and Lowe,1984), bottles diffusing nutrients through a glass fiber filter substrate for periphyton growth (Matlock et al., 1998), and agar-filled vials diffusing nutrients through a wooden or porcelain substrate (Gibeau and Miller, 1989).

Limiting nutrient determination for periphyton growth in an Oklahoma woodland stream was tested by Matlock et al. (1998) with the use of periphytometers, which are floating or anchored racks that hold nutrient diffusing bottles in replicate just under the surface of the water. The nutrients in solution diffused out of a hole cut in the cap of each bottle covered by a dialysis membrane and glass fiber filter. The dialysis membrane was used to have some control of the rate of diffusion and also acted as a biofilter to prevent contamination of the solution in the bottle by algae or bacteria while the glass fiber filter served as a textured surface ideal for periphyton growth. Four nutrient solutions were used: N as 36 mg NO_3-N L^{-1}, P as 20 mg PO_4-P L^{-1}, N+P combined at the same concentrations, and a deionized water control. Ten replicates of each solution were placed in the bottles and submerged in the stream for up to 2 weeks. After deployment, the glass fiber filters were extracted for chlorophyll *a* (chl *a*), which was then

quantified by fluorometry, and used as a measure of algal biomass. They determined that the stream was P-limited. The PO_4-P treatments yielded an average of 3.44 μg chl *a* cm^2, with 1.29 μg chl *a* cm^2 in response to the NO_3-N treatment, and 1.46 μg chl *a* cm^2 in response to the control. Matlock et al. (1999a, 1999b) then used the periphytometer technique to develop a method for determining stream ecosystem trophic status by comparing the baseline periphyton primary production to the maximum potential primary production. Baseline production describes growth in the absence of nutrient addition, while maximum potential production occurs with nutrient enrichment.

The periphytometer has been employed by Carey et al. (2005) for nutrient limitation studies of nine coastal plain rivers in southern Georgia. Treatments used were 87.5 mg L^{-1} NO_3-N, 12 mg L^{-1} PO_4-P, N+P at the same concentrations, and a deionized water control. Chl *a* was quantified by fluorometry. Periphytometer deployments in shaded sites did not measure periphyton growth response to nutrient enrichment. All but three periphytometer deployments in unshaded, high irradiance sites had at least one treatment produce significantly higher chl *a* values when compared to the control. Average chl concentrations ranged between 0.2 and 3.5 μg chl *a* cm^2 for the control treatments and between 1 and 7 μg chl *a* cm^2 for the N, P, or N+P nutrient treatments showing positive periphyton growth response.

Gibeau and Miller (1989) tested for nutrient limitation using nutrient-enriched agar vials containing 390 mg PO_4-P L^{-1} or 13 mg NH_4-N L^{-1}. They covered the openings with porcelain disks and submerged them in an Alaskan river for 3 weeks. Upon retrieval, the disks were removed and analyzed for chl *a* by fluorometry. They determined the river was limited by N+P. Average chl biomass for the N+P treatment was 1.232 μg chl *a* cm^{-2}, for the P treatment was

0.555 μg cm^{-2} chl *a*, for the N treatment was 0.257 μg chl *a* cm^{-2}, and for the control was 0.332 μg chl *a* cm^{-2}.

The objective of this study was to determine if N, P, or both are the limiting nutrients for periphyton growth in streams using both nutrient deionized water-filled bottles and nutrient agar-filled vials in two Georgia Piedmont watersheds, Greenbrier Creek and Rose Creek, located in Greene and Oconee Counties. Land usage in these watersheds is characterized as agricultural and forested. Two types of nutrient-diffusing surfaces were employed as artificial surfaces for algal growth. One consisted of bottles diffusing solutions of N, P, N+P, or a deionized water control through a Durapore membrane and glass fiber filter. The other used vials containing N, P, N+P nutrient enriched agar, or a control agar with no nutrient addition. For purposes of clarity, the bottle/filter system will be referred to as either "bottle" or "filter" and the vial/disk system will be referred to as "vial" or "disk". The bottles and vials were attached to a floating PVC frame, known as a periphytometer (Matlock et al., 1998), and submerged in the streams for 21 to 23 d. The algal biomass on the filters and the porcelain disks were determined by spectrophotometric analysis of chl *a*.

Materials and Methods

Site Description

Eight sites were used for this study, (Figure 3.1) four on each of the Greenbrier and Rose Creeks located in the Georgia Piedmont in Oconee and Greene counties. On Rose Creek, two sites are adjacent to hay fields (G, H), one is forested (F), and one is deforested, planted with grass, and the channel altered with sand and rocks just downstream from a road crossing (E). On

the Greenbrier Creek, one site is adjacent to pasture (B) and three are forested (A, C, D). The cattle from the pasture adjacent to Greenbrier Creek were not allowed in the channel upstream from the study site. The study sites are located at the end of a run and just downstream from a stormflow collector and baseflow sampling sites. All sites have at least 7.5 m of riparian zone, though canopy cover allows direct sunlight at midday with the exception of site A.

Physical Parameter Measurements

At the top of each stormflow collector, located just upstream from a study site, a HOBO microstation (Onset Computer Corporation, Bourne, MA, USA) with sensors for photosynthetically active radiation (PAR) and water temperature was attached. Measurements were collected every minute for the entire duration of each deployment of nutrient-diffusing surfaces in the streams. Dissolved Oxygen (DO) was tested periodically using the handheld YSI 55 DO meter (Yellow Springs Instruments, Yellow Springs, OH, USA).

Historical data for precipitation during periphytometer exposure periods were taken by the weather station at the J. Phil Campbell Sr. Natural Resources Conservation Center, USDA-ARS, located in Watkinsville, Oconee County, Georgia (www.agclimate.org) located between 6 and 23 km from the study sites.

The NO_3-N and PO_4-P concentrations of the stream water (background NO_3-N and PO_4-P) were measured from grab samples taken approximately every two weeks at each site and at locations upstream and downstream from each periphytometer site. When possible, samples from dates up to one month before and after the deployment dates were included in the graphical presentation of the data. The samples were filtered through a 0.45-µm dialysis membrane before analysis. Each solution was analyzed for NO_3-N with the Gries-Ilosvay procedure after

reduction of NO_3^- to NO_2^- (Mulvaney, 1996), and for PO_4-P with the molybdate blue method (Murphy and Riley, 1962).

Nutrient-Diffusing Bottles

The 60-mL narrow-mouth Nalgene bottles (Nalge Nunc International, Rochester, NY, USA) on each periphytometer constituted ten replicates each of a deionized water control, 87 mg NO_3-N L^{-1} (KNO_3 in deionized water), 12 mg PO_4-P L^{-1} (Na_2HPO_4 in deionized water), or 87 mg NO_3-N L^{-1} + 12 mg PO_4-P L^{-1} (KNO_3 + Na_2HPO_4 in deionized water; Carey, 2005). These concentrations are above the maxima set at 0.037 µg P L^{-1} and 0.69 mg N L^{-1} as ambient nutrient concentration for surface waters in Ecoregion IX and below toxic levels for algae growth (USEPA, 2000). A 1.5-cm hole was cut in each cap. The 60-mL bottles were filled to capacity with the N, P, or N+P solution using a repipet, each bottle receiving 63 mL of solution. A 25-mm diameter 934/AH glass fiber filter (Whatman, Middlesex, UK) was cut to 16 mm using a punch and placed inside the lid, covering the whole and held in place by the underside of the lid. A 25-mm diameter, 0.45-µm Durapore dialysis membrane (Millipore, Billerica, MA, USA) was placed on the mouth of the filled 60-mL bottle and the cap with cut glass fiber filter was carefully placed over the membrane and screwed in place. The dialysis membrane was used to have some control of the diffusion and the glass fiber filter acted as a substrate for periphyton growth. Each set of replicates of all three treatments and the control were installed randomly on a periphytometer so that each treatment was represented once in each of ten rows and left for 21 to 24 d at each study site. The bottles were attached to the wire fencing so that the bottle cap surface bearing the filter was perpendicular to the surface of the water.

Nutrient-Diffusing, Agar Vials

Beginning in the spring of 2005, periphytometers carrying agar-filled vials were placed at each site along with the periphytometers carrying nutrient-diffusing bottles (Gibeau and Miller, 1989). Each frame carried 40 30-mL plastic vials with snap tops (Poly-cons, Madan Plastics, Inc., Cranford, NJ). A 1.9-cm hole was cut in the top of each vial and a 2.7-cm porcelain disk was affixed to the opening by heating the disk on a hot plate, allowing it to melt to the surface of the cap, completely covering the hole, and then cooling the disk and cap by dropping them in a beaker of water (Bill Perry, personal communication). Each vial was filled to the top with agar that contained N, P, N+P (15,500 mg PO_4-P L^{-1},7000 mg NO_3-N L^{-1}), or no nutrient addition and then sealed with the cap bearing the fused porcelain disk.

Each agar was prepared by first placing 2 g of powdered agar, granulated solidifying agent (Fisher Scientific), into a 500-mL glass bottle. Then the appropriate amount of KNO_3 and $Na_2H_2PO_4$ was added to a graduated cylinder depending on the treatment in preparation (25.275 g KNO_3 and 29.995 g $Na_2H_2PO_4$) and brought up to 500 mL with deionized water. When the solids were completely dissolved, usually with agitation and heat, the solution was added to the glass bottle with the dry agar. The bottle was placed on a hot plate with a stir bar and heated and stirred until the liquid agar was ready to be poured into the vials. Upon cooling, the tops with fused porcelain disks were snapped into place.

Ten vials of each treatment were placed on the periphytometers in random order. The vials were placed upright so that the surface of the porcelain disk was parallel with the surface of the water. They remained suspended just underneath the surface of the water for 21-24 d at each study site.

Periphytometer

Each periphytometer consisted of a frame constructed with 5-cm diameter PVC pipe. Plastic-coated wire fencing, forming a grid with 5 x 7.5-cm squares, was cut to fit the width and length of the frame and attached to the side that would float just under the surface of the water. Nutrient diffusing bottles or agar-filled vials were attached to the wire fencing so that they would be suspended just under the surface of the water (Matlock et al., 1998).

In-Stream Deployment of Bottle/Filter and Vial/Disk Systems

Filters and disks were prepared as described earlier in the Methods section. Treatments included a control (no nutrient added), N, P, and N+P. Concentrations for N treatments were 78 mg NO_3-N L^{-1} for bottles and 7,000 mg NO_3-N L^{-1} for vials. Concentrations for P treatments were 12 mg PO_4-P L^{-1} and 15,500 mg PO_4-P L^{-1} for vials. Ten replicates of each treatment were randomly placed on two periphytometers, one with bottles and one with vials. The bottles were attached with the filter face perpendicular to the water surface, whereas the vials were attached with the porcelain disk face parallel to the water surface. A periphytometer with bottles and a periphytometer with vials were deployed at eight sites on two streams the spring and summer of 2005. Each periphytometer consisted of a frame constructed with 3-cm diameter PVC pipe. Plastic-coated wire fencing, forming a grid with 5 x 7.5-cm squares, was cut to fit the width and length of the frame and attached to the side that would float just under the surface of the water (Fig 2.4). Periphytometers were exposed to stream flow for 21 to 24 d and then the filters and disks were analyzed for µg chl *a* cm^{-2}.

Collection of the Bottles and Vials

After 21 to 24 d, the periphytometers were removed from the streams and the nutrient-diffusing bottles and agar-filled vials were collected in the field. The solution from each bottle was transferred to scintillation vials which were placed on ice and later stored at 4°C. The glass fiber filters from each bottle cap were placed in zip-loc baggies and placed in the dark on ice until they could be stored at 4°C.

The vial caps with porcelain disks were removed from the agar-filled vials and the agar from each of the vials was placed in a specimen cup and stored at 20°C. Seven disks from each treatment were wrapped in foil and placed on ice until they could be stored at 4°C. The remaining three randomly chosen disks from each treatment were submersed in diluted formalin solution (approximately 1% formalin) in specimen cups and stored at room temperature for later algal taxonomic analysis.

Sample Preparation for Chl a *Extraction*

The glass fiber filters collected from the nutrient-diffusing bottles were removed from the freezer and in low-light conditions each was placed in a 2-mL microcentrifuge tube. The remaining volume of the centrifuge tube was filled with 1.95 to 1.97 mL of 90% ethanol. Each tube was placed in a water bath at 78°C for 5 min then stored at 20°C overnight. The following day, the tubes were centrifuged at 3000 rpm for 5 min and the supernatant was analyzed using a spectrophotomer (MicroQuant, Bio-Tek Instruments, Inc., Winooski, VT, USA) as described below.

The chl *a* from the porcelain disks fused to the vial caps was extracted in 10 mL of 90% ethanol in a specimen cup. They were placed in the water bath at 78°C for 5 min and stored at

20°C overnight. The following day, 2 mL of solution from each disk extraction was pipetted and placed in a 2-mL microcentrifuge tube. The microcentrifuge tubes were then centrifuged at 3000 rpm for 5 min and the supernatant was analyzed using a spectrophotomer (MicroQuant, Bio-Tek Instruments, Inc., Winooski, VT, USA) as described below.

Preparation for Spectrophotometry

A chl *a* standard was prepared to test for the optimal spectrophotometric wavelength for sample analysis. The optimal wavelength is that which is maximally absorbed when passed through the sample. Concentrated chl *a* from *Anacystis nidulans* algae (Sigma-Aldrich Company) in the powder form was dissolved in 90% ethanol to make a solution of 20 mg chl *a* L^{-1}. It was boiled in a water bath at 78°C for 5 min and stored overnight at 20°C. The chl *a* solution was centrifuged at 3000 rpm for 5 min. Then dilutions of 100, 200, 400, and 800 μg L^{-1} were performed to be used as standards. Using the 400 μg L^{-1} chlorophyll *a* standard, 24 wells of a 96-well, flat-bottomed plate were filled to 0.2 mL and analyzed at wavelengths between 660 and 665 nm, as the absorbance peak for chl *a* always falls between these two wavelengths (Biggs and Kilroy, 2000). The peak absorbance was determined to occur at a wavelength of 664 nm.

Chl a *Analysis by Spectrophotometry*

For spectrophotometry, the centrifuged samples of both the filter and disk extractions were pipetted from the 2-mL microcentrifuge tubes into a 96-well, flat-bottomed plate. For each sample, two wells were filled to a volume of 0.2 mL with the chl *a* extract. Every prepared plate included 4 wells of 90% ethanol filled to 0.2 mL as a blank. The spectrophotometer was set to take readings at 664 nm and 750 nm and the plate was read three times. Then, 0.05 mL of 0.3 M

HCl was added to each well and, after 30 s, readings at 664 nm and 750 nm were again recorded three times.

The difference in absorbances recorded at the two wavelengths were calculated and averaged, both before and after the HCl addition. The average absorbance difference from before and after the HCl addition were inserted into a formula to determine μg chl *a* in the sample (Biggs and Kilroy, 2000). The absorbance readings were first corrected for wavelength distance to account for different volumes of sample before and after acidification. The μg chl *a* cm^{-2} was then determined using the following equation:

Chlorophyll *a* (μg cm^{-2}) = [($abs_{664\ before} - abs_{750\ before}$) – ($abs_{664\ after} - abs_{750\ after}$) x 28.66 μg L^{-1} x sample volume]/area of filter or disk

The corrected absorbance readings at 750 nm before and after acidification are subtracted from the corrected absorbance readings at 664 nm before and after acidification. 28.66 μg mL^{-1} is the absorption coefficient for chl *a* as defined by Sartory and Grobbelaar (1984).

The amount of chl *a* on the filters and disks was then determined and expressed as μg chl *a* cm^{-2}. The area of the glass fiber filters was determined to be 1.767 cm^2 and the area of the porcelain disk was 5.726 cm^2.

Determination of final N and P Concentrations of the Bottle Solutions

The solution collected from the nutrient diffusing bottles was allowed to thaw after removal from storage at 4°C. Each solution was analyzed for NO_3-N with the Gries-Ilosvay procedure after reduction of NO_3^- to NO_2^- (Mulvaney, 1996), and for PO_4-P with the molybdate blue method (Murphy and Riley, 1962).

Determination of final N and P Concentrations of the Agars

Each agar was removed from the vial and extracted by the same method. Using a hand-mixer, the agar was ground for 3 s, placed in 800 mL of deionized water, and shaken for 2 h. Then, the extractant solutions were tested for NO_3-N with the Gries-Ilosvay procedure after reduction of NO_3^- to NO_2^- (Mulvaney, 1996), and for PO_4-P with the molybdate blue method (Murphy and Riley, 1962).

Phosphorus Trophic Status (PTS)

The Phosporus Trophic Status (PTS) of each site during each deployment was calculated by dividing the average chl *a* concentration in the control treatment by the average chl *a* concentration in the P treatment. These PTS ratios were plotted against stream background PO_4-P concentrations (for the deployment period) to determine their relationship for spring and summer 2005.

Statistical Analysis

Comparisons of chl *a* concentrations were made using generalized linear model (glm) statistical analyses using SAS statistical software (SAS Institute, Cary, North Carolina) in a hierarchical fashion. First, comparisons of chl *a* concentrations were made between streams (Greenbrier and Rose), which included media (bottles and vials) and treatment data, by deployment date (seasons). Then comparisons were made between sites (A, B, C, D, E, F, G, and H), by media and by season. Subsequently, comparisons between treatments (N, P, N+P, and control) were made by media and by site.

Results and Discussion

Physical Parameter Measurements

PAR ranged between 19 mole photons m^{-2} and 221 mole photons m^{-2} (Tables 3.1 and 3.2) Generally, the low values ranged between 19 and 56 mole photons m^{-2} and the high values ranged between 110 and 221 mole photons m^{-2}. The HOBO microstations became damaged later in the experiment due to heavy rains and stream flooding during storm events in the spring of 2005. Therefore, environmental data were not collected for the 2005 deployments of the periphytometers (Tables 3.1 and 3.2; Fig 3.2).

The mean water temperatures during the spring and summer months ranged between 19°C and 25°C. During the fall months, the mean water temperatures ranged from 12°C to 15°C.

The average values for background PO_4-P were consistently below the 0.03 mg L^{-1} standard set by the USEPA (2000) at sites A, D, E, F, G, and H (Figs. 3.3 through 3.6). Measurements at these sites ranged from 0.005 mg PO_4-P L^{-1} to 0.025 mg PO_4-P L^{-1}. At sites C and B, the background PO_4-P concentrations were above the 0.03 mg PO_4-P L^{-1} standard for all or part of the study period. Site C average PO_4-P background concentration was 0.09 mg PO_4-P L^{-1} in the summer of 2004, 0.111 mg PO_4-P L^{-1} in the fall of 2004, 0.064 mg PO_4-P L^{-1} in the spring of 2005, and 0.077 PO_4-P mg L^{-1} in the summer of 2005. Although the average PO_4-P background concentration for site B is below the 0.03 mg PO_4-P L^{-1} standard, it did rise above the standard in the spring and summer of 2005.

The average values for background NO_3-N were consistently above the 0.69 mg N L^{-1} standard set by the USEPA (2000) at sites A, B, E, F, G, and H (Figs. 3.7 through 3.10). Only sites C and D were consistently below the 0.69 mg NO_3-N L^{-1} standard but, with average

measurements between 0.37 mg NO_3-N L^{-1} and 0.61 mg NO_3-N L^{-1} they were close and at times over the 0.69 mg N L^{-1} standard. Franklin et al. (2002) showed that average NO_3-N concentrations at sites on the Rose Creek in 1999 and 2000 were significantly greater than NO_3-N concentrations in Greenbrier Creek ($p<0.0001$).

Nutrient Diffusion

At the end of the exposure period, P-treated bottles retained between 21% and 72 % of the initial PO_4-P (Tables 3.3 and 3.4). The flux of PO_4-P through the filters ranged from 0.50 mg PO_4-P cm^{-2} to 0.63 mg PO_4-P cm^{-2} for the exposure period. At the end of the exposure period, N-treated bottles retained between 4% and 21 % of the initial NO_3-N (Tables 3.3 and 3.4). The flux of NO_3-N through the filters ranged from 4.6 mg NO_3-N cm^{-2} to 0.5.9 mg NO_3-N cm^{-2} for the exposure period.

In contrast, at the end of the exposure period P-treated vials retained between 36% and 78 % of the initial PO_4-P (Tables 3.4). The flux of PO_4-P through the disks ranged from 0.31.7 mg PO_4-P cm^{-2} to 65.2 mg PO_4-P cm^{-2} for the exposure period. At the end of the exposure period, N-treated vials retained between 21% and 95 % of the initial NO_3-N (Tables 3.4). The flux of NO_3-N through the disks ranged from 7.8 mg NO_3-N cm^{-2} to 34.9 mg NO_3-N cm^{-2} for the exposure period. The P flux from disks is about 100 times greater than for filters and the N flux is about 4 times greater than for filters.

Treatment Comparisons of Chl a *Concentrations*

Statistical analysis showed a significant difference ($p<0.05$) for bottle and vial chl *a* concentrations between the Greenbrier and Rose Creeks (Fig 3.11) Significant differences were also indicated for sites, media (bottle or vial), and deployment ($p<0.05$). Therefore, in general, treatment differences in chl *a* concentrations will be presented by stream, media, deployment (season), and site. Agar vials were only deployed in 2005.

Comparison of Streams (across all treatments)

Chl *a* concentrations across media were significantly greater for Rose Creek (0.35 μg chl *a* cm^{-2} to 4.18 μg chl *a* cm^{-2}) than for Greenbier Creek (0.06 μg chl *a* cm^{-2} to 1.16 μg chl *a* cm^{-2}) for periphytometer deployments in every season, except for the lower reaches in fall 2004 and the upper reaches in summer 2005 when no significant differences between streams were found (Fig 3.11).

Comparisons of Sites by deployment (for nutrient-free bottles and vials)

To compare productivity (production of chl *a*) between sites in which bottles and vials were deployed at the same time, we analyzed differences between chl *a* concentrations on control treatments (nutrient-free bottles and vials). Sites that were tested at the same time were those in lower reaches (C, D, G, and H) followed by those in the upper reaches (A, B, E, and F). It should be noted that vials were only deployed in 2005. For nutrient-free bottles, we found that sites A, C, D, and F always produced significantly smaller chl *a* concentrations ($p=0.05$; Tables 3.1 and 3.2). Where bottles were deployed in the lower reaches for summer 2004 and spring and

summer 2005, sites G and H always had significantly more chl *a* productivity (Tables 3.1 and 3.2).

In spring and summer of 2005, no significant differences in chl *a* productivity were indicated for vials deployed in the lower reaches of both streams ($p=0.05$). In the upper reaches, sites B and E were significantly more productive than sites A and F (Table 3.2).

Comparison of Nutrient-Diffusing Bottles

As indicated in the methods section, seasonal deployments were done in two sets, or time periods. Lower reaches (sites C, D, G, and H) were deployed at separate times than upper reaches (sites A, B, E, and F). This was done to accommodate equipment and time restrictions.

The only significant difference in filter chl *a* concentration between nutrient-diffusing bottle treatments within a site was measured at site E during the spring of 2005. (Fig. 3.12) The N+P treatment produced significantly greater chl *a* than the N, P, or control treatments and the N treatment produced significantly greater chl *a* concentration than either the control or the P treatments. At site E, background stream NO_3-N concentrations were above 0.7 mg NO_3-N L^{-1} and background PO_4-P concentrations were below 0.04 mg PO_4-P L^{-1} (Table 3.2, Figs 3.5 and 3.9). Given these background stream nutrient concentrations, one would expect this site to be P-limited. There is at least minimal evidence to indicate that this site is N-limited in that the addition of NO_3-N increased periphyton growth.

Comparison of Nutrient-Diffusing, Agar Vials

Differences between treatments within sites were found using the agar vial method only for deployments in the spring of 2005 (Figs. 3.12-3.19). In the upper reaches of Greenbrier Creek, site B chl *a* concentrations on the P-treated vials were significantly greater than the N, N+P, and the control treatments, which were not significantly different from each other (Fig. 3.12). In the lower reaches of Greenbrier Creek, at site D chl *a* concentrations from the P-treated vials were significantly greater than the N, N+P, and the control treatments. The N+P treatment, however, was significantly greater than the N and control treatments (Fig. 3.13).

In the upper reaches of the Rose, at site E, the P and N+P treatment chl *a* concentrations were not significantly different from each other, but were significantly greater than the control treatment chl *a* concentration (Fig. 3.14). The P treatment was not significantly greater than the N treatment. In the lower reaches of the Rose, at site G, P and N+P treatments had significantly greater chl *a* concentration than the control and were not significantly different form each other (Fig. 3.15). At site H, none of the treatments produced significantly different chl *a* concentrations from the control (Fig. 3.15). However, the P treatment chl *a* concentration was significantly greater than that of the N and N+P treatments.

Four out of seven sites in spring 2005 were P-limited and light did not appear to be a limitation (PAR high; Table 3.2). None of the seven sites was N-limited for the agar-vials (Figs. 3.12-3.15). This differed from the bottles, where one of the seven sites (site E, Fig. 3.14) was N-limited and PAR was low when compared to that of the P-limited streams (Table 3.2).

Phosphorus Trophic Status (PTS)

The PTS was calculated for each site and deployment time in 2005 (Fig. 3.20). Results indicated that in the spring of 2005, where P-limitation was found at 4 out of the 7 sites, background stream P concentrations ranged between 0.01 mg PO_4-P L^{-1} and 0.03 mg PO_4-P L^{-1} and the PTS was equal to or below 0.6. Work conducted in Texas showed that P-responsive sites had Lotic Ecosystem Trophic Status (LETSI) of 0.5 or less (McFarland et al., 2004). The LETSI is calculated by dividing chl *a* for the control treatment by the chl *a* in the N + P treatment. In this study, we choose a ratio between chl *a* for the control over the chl *a* for the P treatment because we observed inhibition of chl *a* production in some of the N treatments (Site D, Fig. 3.15; and Site H Fig. 3.13).

In the summer of 2005, chl *a* concentration on the disks was greater than in the spring of 2005, although no P-limitation was found and the majority of sites had PTS values above 0.6. This indicates that PTS may be a useful tool to identify responsive sites, but the poor relationship with stream PO_4-P concentration ($r = 0.09$; $p<0.75$) suggests other variables should be considered when developing a tool for predicting P response based on a threshold PTS.

The different response to treatments in the spring and summer 2005 could be because of differences in the amount of PAR that the algal-growing media received in the spring and summer. Although total PAR values (measured at J. Phil Campbell Sr. Natural Resources Conservation Center, USDA-ARS, located between 6 and 23 km from the study sites) were very similar between deployment periods (564 mole photon m^{-2} for spring and 566 mole photon m^{-2} for summer), the total rain received during deployment was 43.8 cm for spring and 11.3 cm for summer. The larger amount of rain received during the spring period probably maintained a greater level of turbidity in the streams which would have reduced the PAR to which the algal-

growing media was exposed. A possible explanation for the lack of response to P in the summer 2005 could be provided by seasonal changes in periphyton population densities and community structure. Additional research should be conducted to determine periphyton dynamics in Southern Piedmont streams.

Conclusions

Using the nutrient-diffusing bottles, only one site (site E) out of seven was limited by N in spring 2005. The NO_3-N concentration in the stream was 2.9 mg L^{-1}. The lack of P response with the filters could have been caused by the P flux through the filter which was about 100-fold less than the flux through the disks. In contrast, P-limitation was indicated for four sites (sites E, G, B, and D) out of seven in spring 2005 when using nutrient-diffusing agar vials. The PO_4-P concentration in the responsive sites ranged from 0.01 to 0.03 mg P L^{-1}. In the summer, the same sites did not show a response to P enrichment. Possible explanations for the different response to treatments in the spring and summer may be different amounts of PAR received by the growing surface and possible seasonal changes in periphyton community structure.

The Phosphorus Trophic Status, calculated as the ratio of chl *a* concentration on the control over the chl *a* concentration on the P-treated vials, showed that P-limited sites in spring 2005 had PTS values at or below 0.6. In the summer 2005, none of the sites responded to P treatment and PTS values were at or above 0.6. This suggests that it may be possible to identify a threshold PTS value below which a P response, as indicated by chl *a*, would be expected. If such a PTS threshold is identified, it would be useful to explore the possibility of estimating PTS for a stream from environmental variables. In this study, no strong relationship was found between

PTS and stream PO_4-P concentration, indicating that additional work should be conducted to explore other variables that may be strongly related to PTS.

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Table 3.1. Summary of stream physical parameters for each site during summer and fall 2004.

Reaches	Stream	Site	Exposure time	Total Photosynthetically Active Radiation	Mean Temperature	Stream PO_4-P	Stream NO_3-N	Mean Chl *a,* controls, bottles
			d	mole photons m^{-2}	°C	----------mg L^{-1} ----------		µg cm^{-2}
				summer (July 2 – August 17)				
Upper	Greenbrier	A	21	22	22.5	0.011	1.37	0.12a†
		B	21	221	22.1	0.018	0.34	0.14a
	Rose	E	21	117	21.6	0.006	2.97	0.55b
		F	21	27	22.3	0.01	1.88	0.14a
Lower	Greenbrier	C	21	130	22.5	0.09	0.45	0.06a
		D	21	110	25.8	0.006	0.48	0.00a
	Rose	G	21	32	22.3	0.005	1.30	1.82b
		H	21	28	22.7	0.008	0.66	2.93c
				fall (November 19 – December 10)				
Upper	Greenbrier	C	22	169	12.6	0.111	0.61	0.54a
		D	22	124	12.4	0.007	0.37	0.26a
	Rose	G	22	29	14.5	0.006	1.40	1.38a
		H	22	143	12.9	0.011	0.76	0.11a

† Within a column, reach, and deployment, means followed by the same letter are not significantly different according to Fisher's LSD at p=0.05.

Table 3.2. Summary of stream physical parameters for each site during spring and summer 2005.

Reaches	Stream	Site	Exposure time	Total Photosynthetically Active Radiation	Mean Temperature	Stream PO_4-P	Stream NO_3-N	Mean Chl *a*, controls, agar vials	Mean Chl *a*, controls, bottles
			d	mole photons m^{-2}	°C	------mg L^{-1} ------		-------µg cm^{-2}------	
				spring (May26 – July 13)					
Upper	Greenbrier	A	24	118	22.9	0.010	1.35	0.12a†	0.09a†
		B	24	no data	no data	0.025	1.05	2.58b	1.25a
	Rose	E	24	56	20.4	0.011	2.90	4.59c	2.04a
Lower	Greenbrier	C	22	133	19.6	0.064	0.54	3.44a	0.58a
		D	22	134	21.4	0.009	0.56	2.03a	0.16a
	Rose	G	22	no data	no data	0.011	1.64	2.84a	1.36b
		H	22	19	19.2	0.017	0.87	3.29a	1.07b
				summer (August 3 – September 19)					
Upper	Greenbrier	A	22	no data	no data	0.009	1.49	1.59a	0.08a
		B	22	no data	no data	0.023	1.03	12.06c	2.09c
	Rose	E	22	no data	20.3	0.009	2.28	8.27b	0.78b
		F	22	35	21.4	0.010	1.76	0.59a	0.13a
Lower	Greenbrier	C	24	no data	no data	0.077	0.40	5.88a	0.07a
		D	24	no data	no data	0.007	0.44	1.95a	0.06a
	Rose	G	24	no data	no data	0.009	1.51	2.76a	0.47b
		H	24	no data	no data	0.012	0.75	5.07a	1.84c

† Within a column, reach, and deployment, means followed by the same letter are not significantly different according to Fisher's LSD at p=0.05.

Table 3.3. Summary of diffused and final nutrient concentrations from bottles deployed in summer and fall 2004.

Stream	Site	Exposure time	Flux of PO_4-P through filters	Flux of NO_3-N through filters	Final PO_4-P in bottles†	Final NO_3-N in bottles‡
		d	----------mg cm^{-2}----------		----------mg----------	
		summer (July 2 – August 17)				
Greenbrier						
	A	21	0.57	4.94	0.29 (40)	0.49 (9)
	B	21	0.53	4.79	0.34 (47)	0.76 (15)
	C	21	0.59	5.02	0.22 (31)	0.36 (7)
	D	21	0.58	5.03	0.25 (35)	0.34 (7)
Rose						
	E	21	0.59	4.95	0.23 (32)	0.48 (9)
	F	21	0.57	4.99	0.27 (37)	0.41 (8)
	G	21	0.61	5.08	0.19 (26)	0.25 (5)
	H	21	0.55	5.02	0.29 (40)	0.35 (7)
		fall (November 19 – December 10)				
Greenbrier						
	C	22	0.50	4.97	0.39 (54)	0.44 (8)
	D	22	0.57	4.81	0.26 (36)	0.73 (14)
Rose						
	G	22	0.58	4.72	0.25 (35)	0.88 (17)
	H	22	0.55	5.89	0.31 (43)	0.58 (11)

† The initial amount of PO_4-P in each bottle was 0.72 mg. Numbers in parentheses are percentages of initial amounts.

‡ The initial amount of NO_3-N in each bottle was 5.22 mg. Numbers in parentheses are percentages of initial amounts.

Table 3.4. Summary of diffused and final nutrient concentrations from bottles and agars deployed in spring and summer 2005.

Stream	Site	Exposure time	Flux of PO_4-P through filters	Flux of NO_3-N through filters	Final PO_4-P in bottles†	Final NO_3-N in bottles‡	Flux of PO_4-P through disks	Flux of NO_3-N through disks	Final PO_4-P in agars¶	Final NO_3-N in agars#
		d	------mg cm^{-2}-------		--------mg--------		-------mg cm^{-2}-------		--------mg--------	
			spring/early summer (May26 – July 13)							
Greenbrier										
	A	24	0.60	5.09	0.21 (29)	0.18 (3)	45.3	21.1	282.9 (61)	124.4 (59)
	B	24	0.57	4.89	0.26 (36)	0.58 (11)	41.2	13.2	306.5 (66)	169.4 (80)
	C	22	0.61	5.03	0.19 (26)	0.34 (7)	35.6	18.1	339.0 (73)	141.3 (67)
	D	22	0.62	5.03	0.17 (24)	0.34 (7)	31.8	11.9	288.6 (62)	176.7 (84)
Rose										
	E	24	0.42	4.62	0.52 (72)	1.07 (20)	55.8	23.1	223.1 (48)	114.2 (83)
	G	22	0.61	5.00	0.19 (26)	0.39 (7)	44.1	13.1	290.4 (62)	182.7 (72)
	H	22	0.61	5.03	0.20 (28)	0.33 (6)	39.7	7.8	315.1 (68)	200.1 (95)
			late summer/fall (August 3 – September 19)							
Greenbrier										
	A	22	0.57	4.68	0.23 (32)	0.95 (18)	34.7	12.2	349.0 (75)	175.0 (83)
	B	22	0.60	4.92	0.22 (31)	0.52 (10)	38.6	16.3	321.3 (69)	151.5 (72)
	C	24	0.63	5.09	0.17 (24)	0.22 (4)	61.1	30.1	192.4 (41)	72.5 (35)
	D	24	0.62	5.09	0.18 (25)	0.22 (4)	65.2	34.9	169.0 (36)	45.0 (21)
Rose										
	E	22	0.60	4.60	0.22 (31)	1.09 (21)	36.7	10.6	332.4 (71)	184.4 (88)
	F	22	0.61	5.01	0.20 (28)	0.36 (7)	31.7	21.3	361.2 (78)	123.0 (59)
	G	24	0.63	5.11	0.15 (21)	0.20 (4)	38.7	17.5	324.1 (70)	145.0 (69)
	H	24	0.58	5.11	0.24 (33)	0.19 (4)	34.5	23.2	345.0 (74)	130.9 (62)

† The initial amount of PO_4-P in each bottle was 0.72 mg. Numbers in parentheses are percentages of initial amounts.
‡ The initial amount of NO_3-N in each bottle was 5.22 mg. Numbers in parentheses are percentages of initial amounts.
¶ The initial amount of PO_4-P in each agar was 465 mg. Numbers in parentheses are percentages of initial amounts.
# The initial amount of NO_3-N in each agar was 210 mg. Numbers in parentheses are percentages of initial amounts.

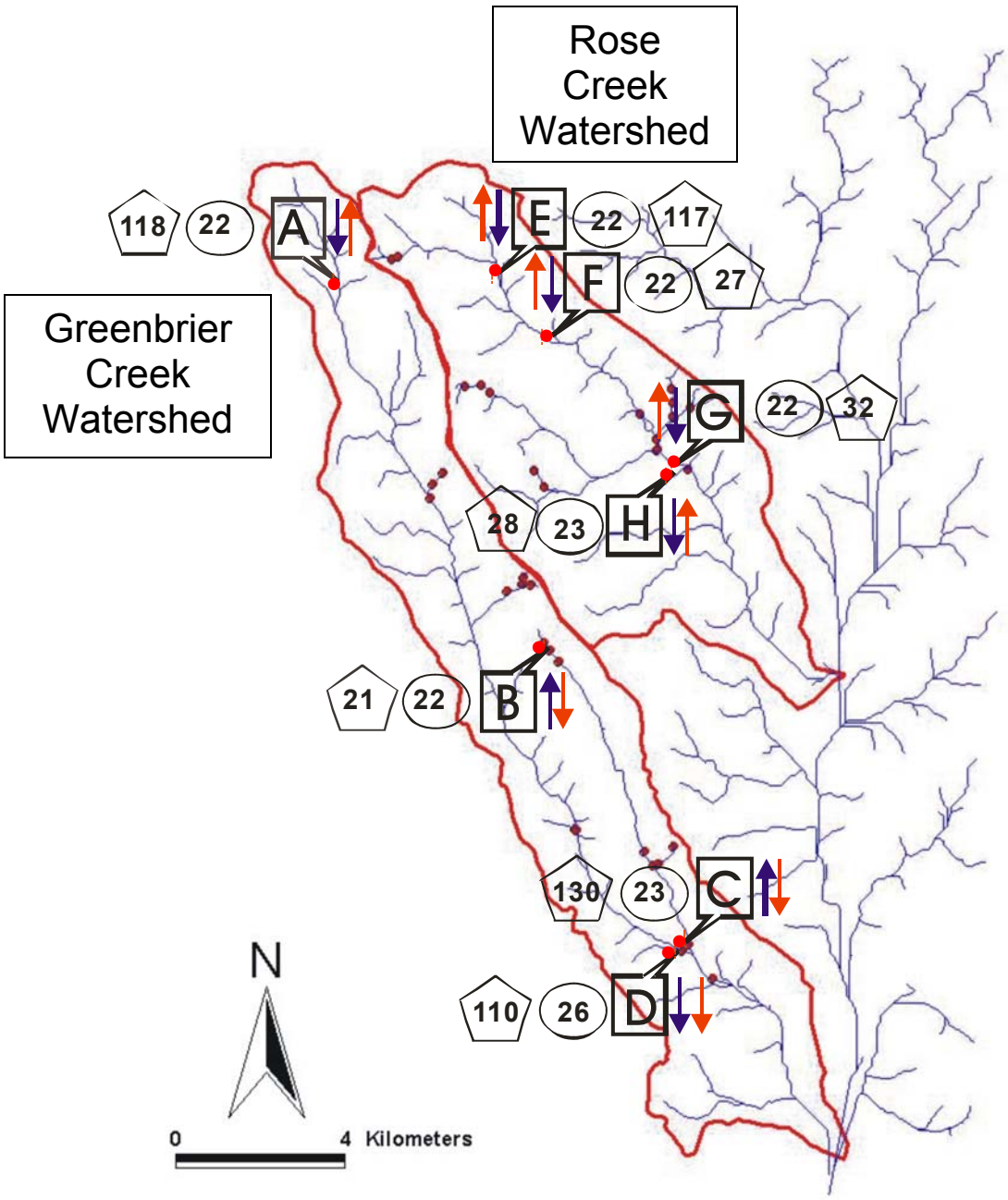

Figure 3.1. Map of Greenbrier and Rose Creek watersheds located in Oconee and Greene Counties, Georgia. Four sampling sites on each watershed. Average temperature in circle. Average PAR in pentagon. Blue arrow is stream background P concentration. Red arrow is stream background N concentration. Arrows pointing up denote concentrations above the EPA guidelines. Arrows pointing down denote concentrations below the EPA guidelines.

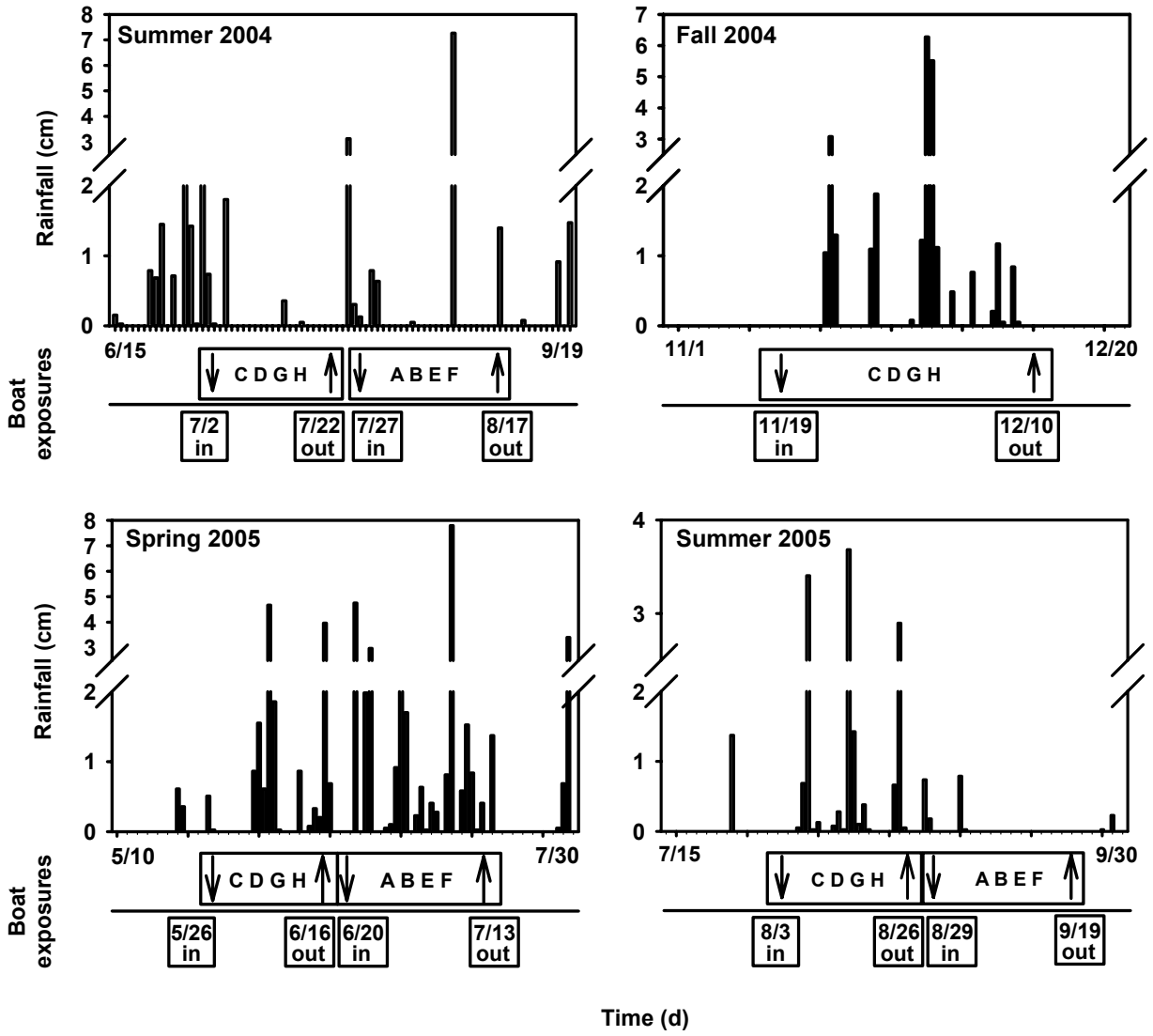

Figure 3.2. Rainfall during periphytometer exposure periods. Boxes below the horizontal axis symbolize periods in which periphytometers were installed at each site. The letters inside each box denote sites in which periphytometers were present. Installation and removal dates are shown in boxes below the lower horizontal axis. In each season, the sites in the lower reaches of each stream (CDGH) were sampled first and the upper reaches (ABEF) were sampled second (except for fall 2004, when only the lower reaches were sampled).

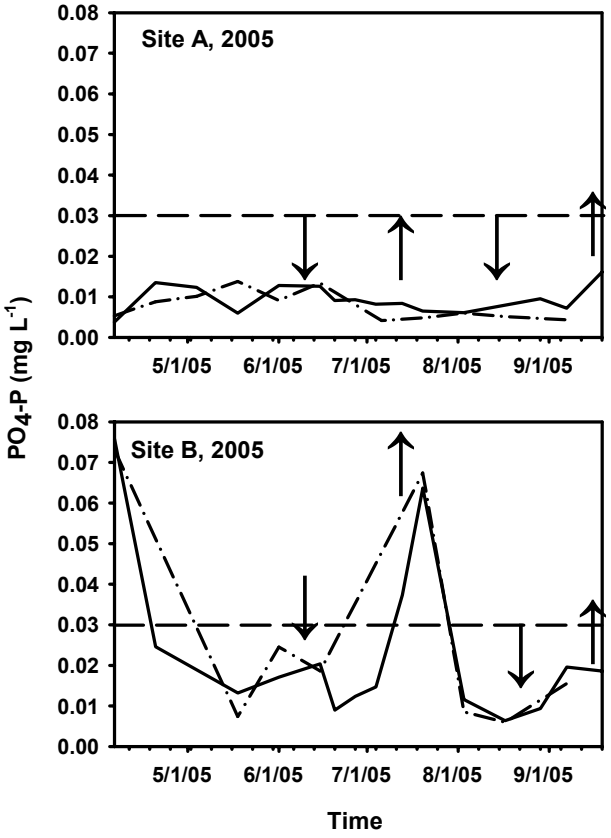

Figure 3.3. Background PO_4-P concentrations for Greenbrier Creek, upper reaches, from May 2005 to September 2005 (no data for 2004). EPA PO_4-P concentration guideline (0.03 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

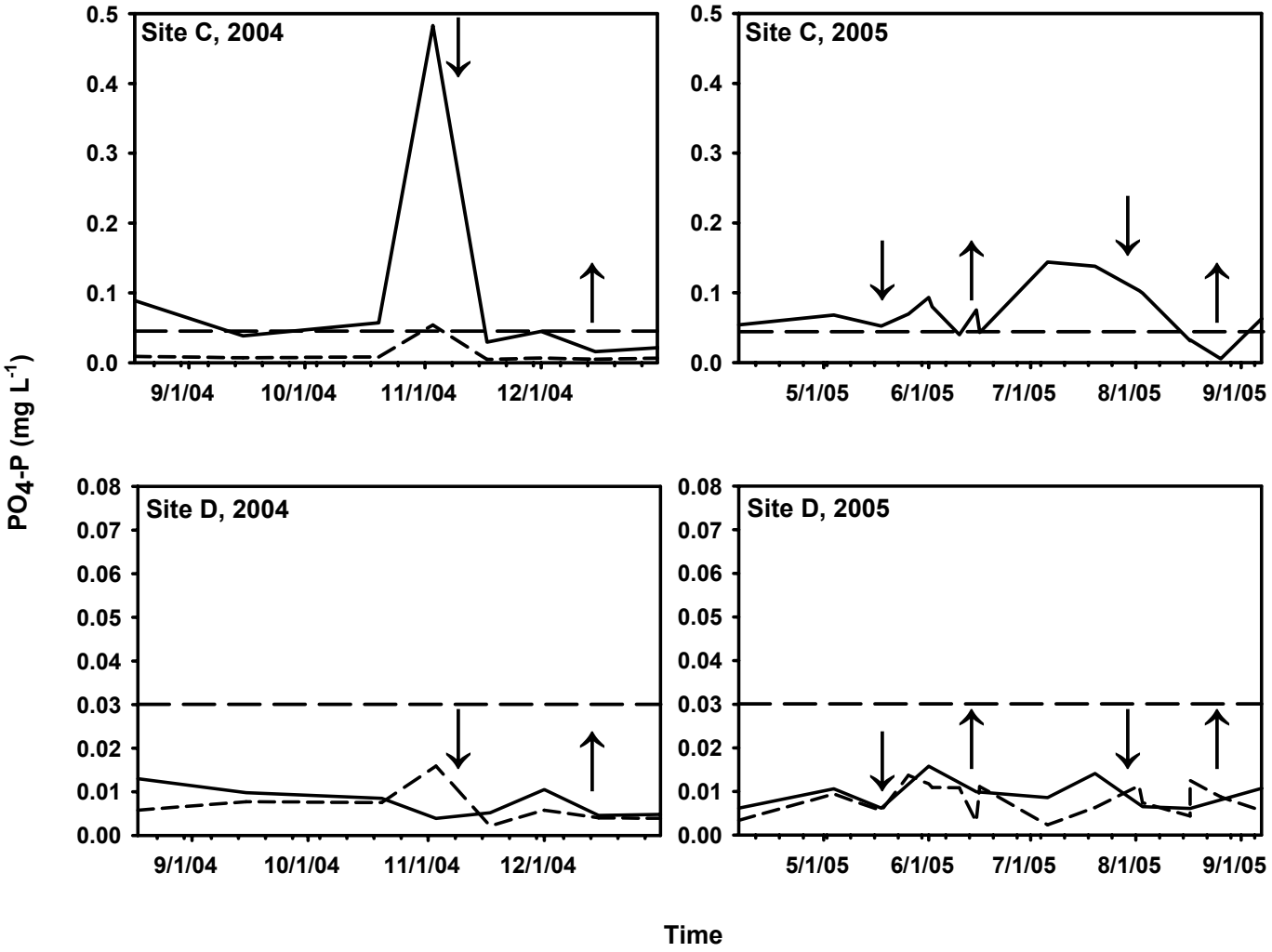

Figure 3.4. Background PO_4-P concentrations for Greenbrier Creek, lower reaches, from September 2004 to September 2005. EPA PO_4-P concentration guideline (0.03 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

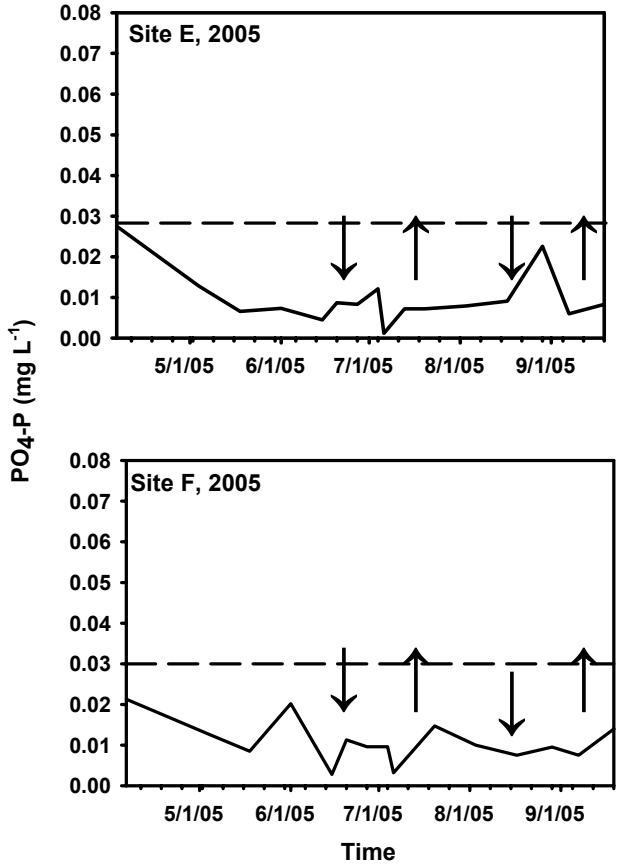

Figure 3.5. Background PO_4-P concentrations for Rose Creek, upper reaches, from May 2005 to September 2005. EPA PO_4-P concentration guideline (0.03 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

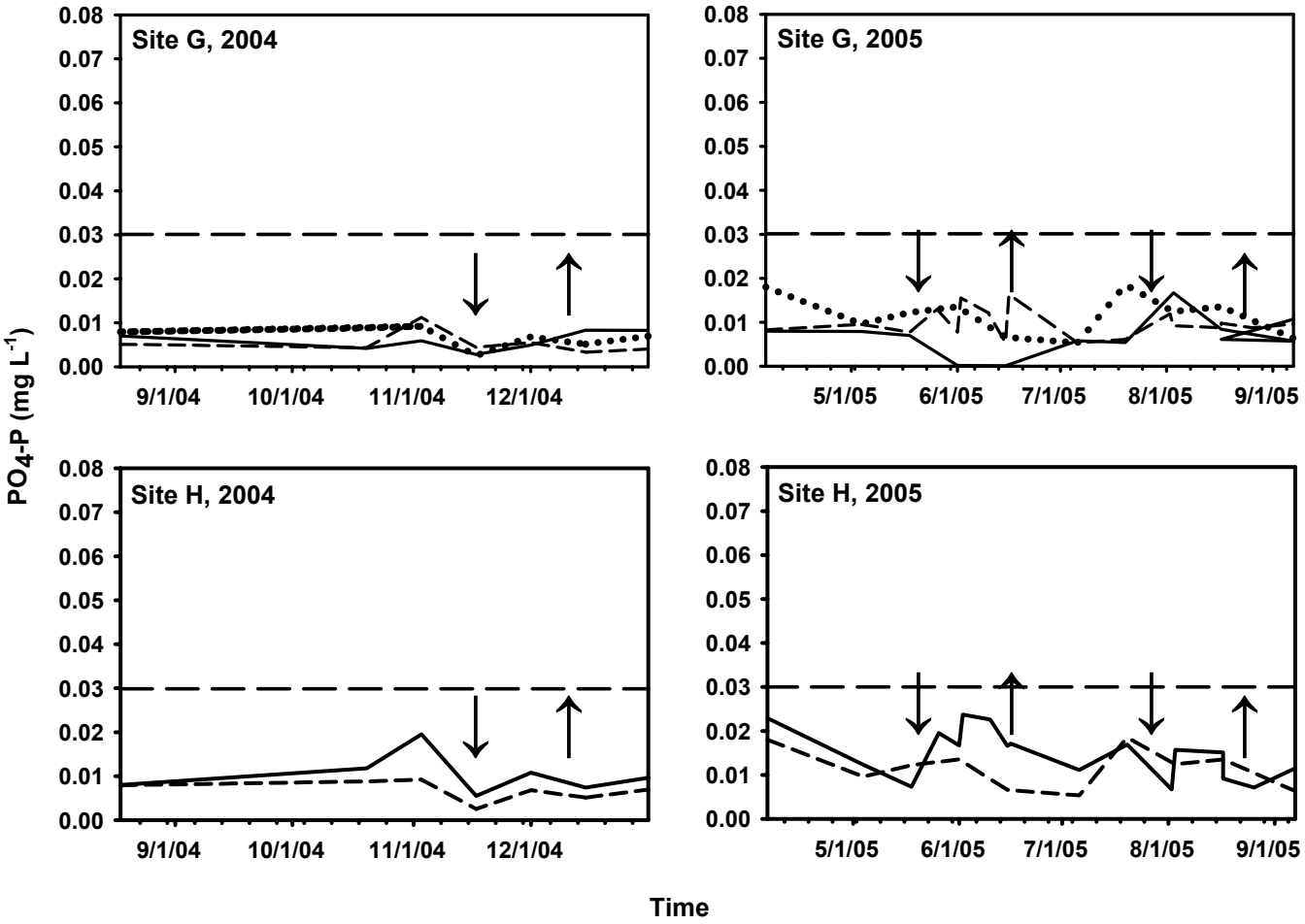

Figure 3.6. Background PO_4-P concentrations for Rose Creek, lower reaches, from September 2004 to September 2005. EPA PO_4-P concentration guideline (0.03 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Dark-dotted lines are concentrations at sampling locations further upstream. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

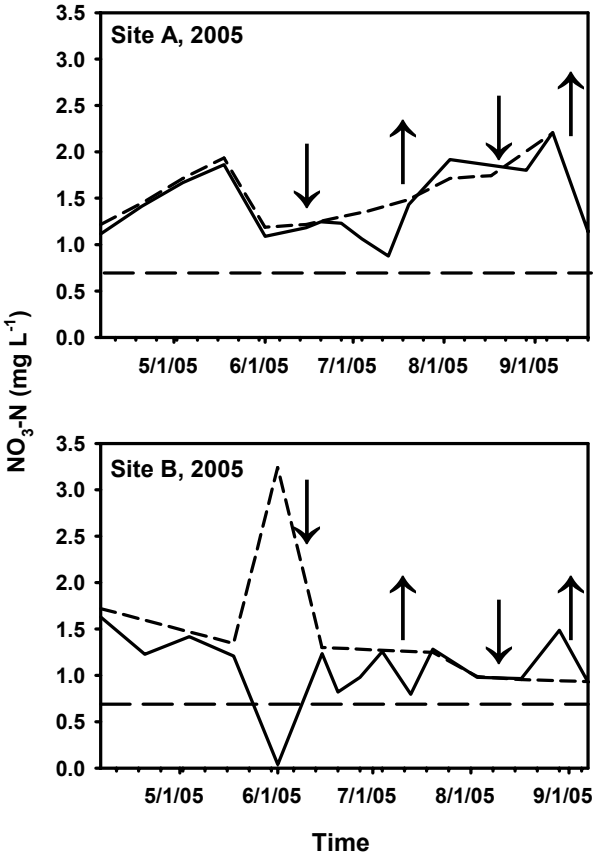

Figure 3.7. Background NO_3-N concentrations for Greenbrier Creek, upper reaches, from May 2005 to September 2005. EPA NO_3-N concentration guideline (0.69 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

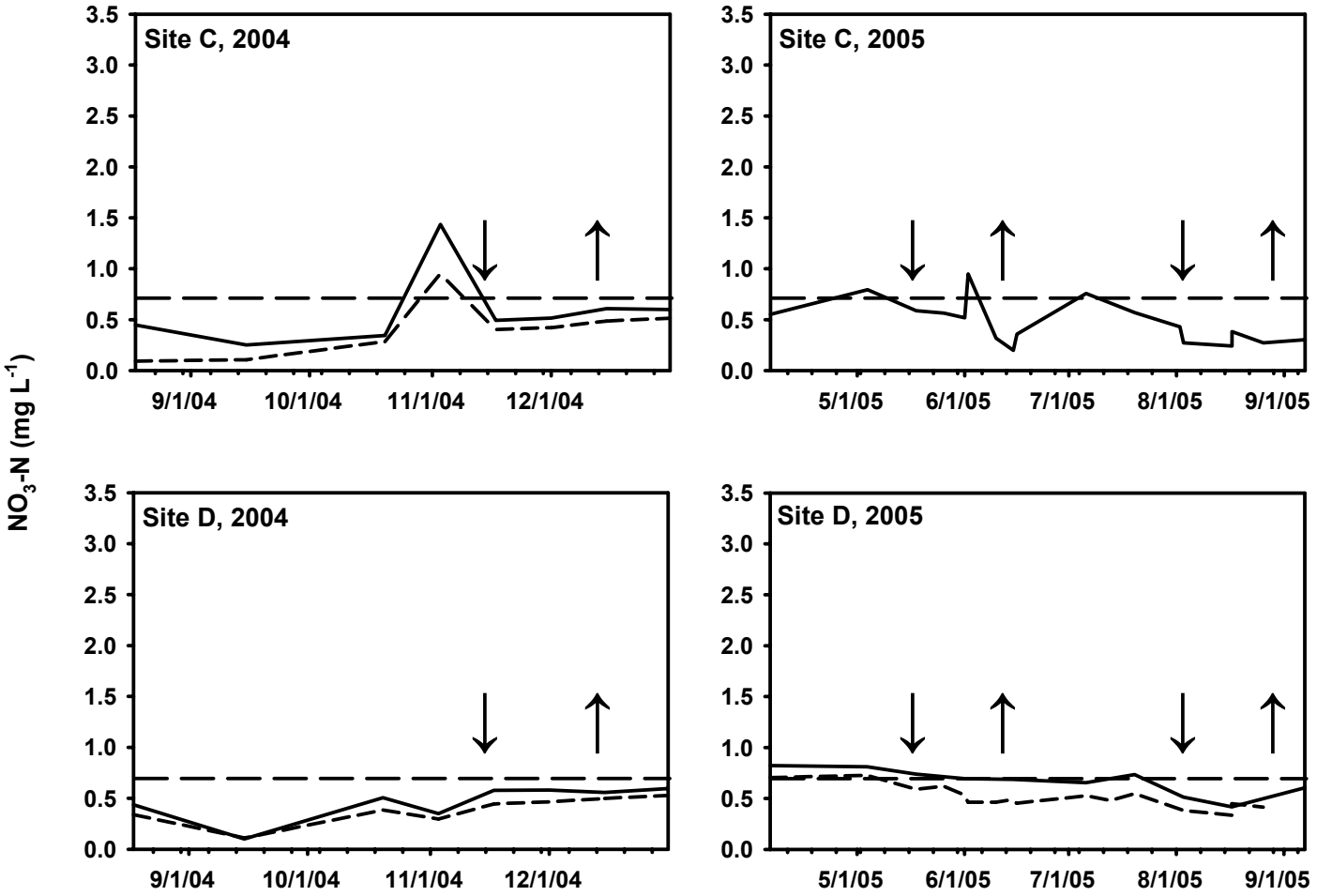

Figure 3.8. Background NO_3-N concentrations for Greenbrier Creek, lower reaches, from September 2004 to September 2005. EPA NO_3-N concentration guideline (0.69 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

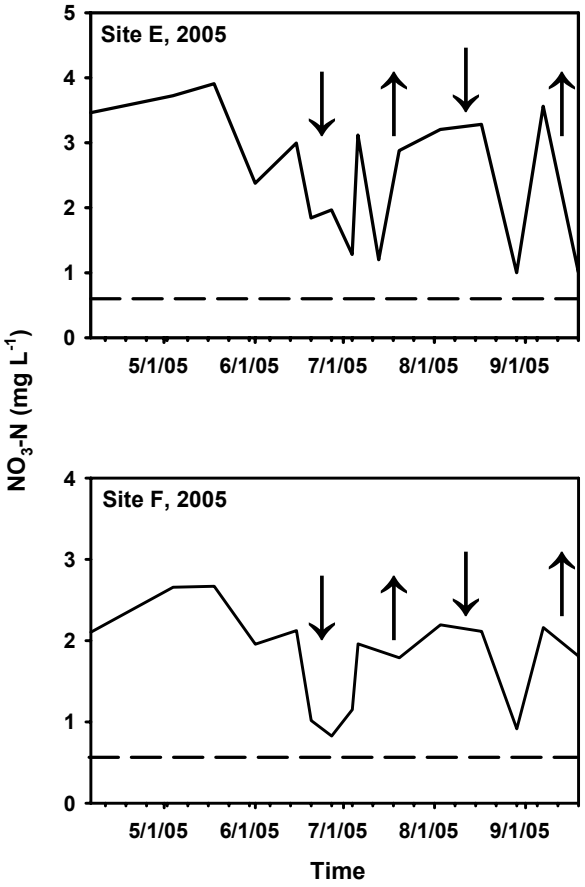

Figure 3.9. Background NO_3-N concentrations for Rose Creek, upper reaches, from May 2005 to September 2005. EPA NO_3-N concentration guideline (0.69 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

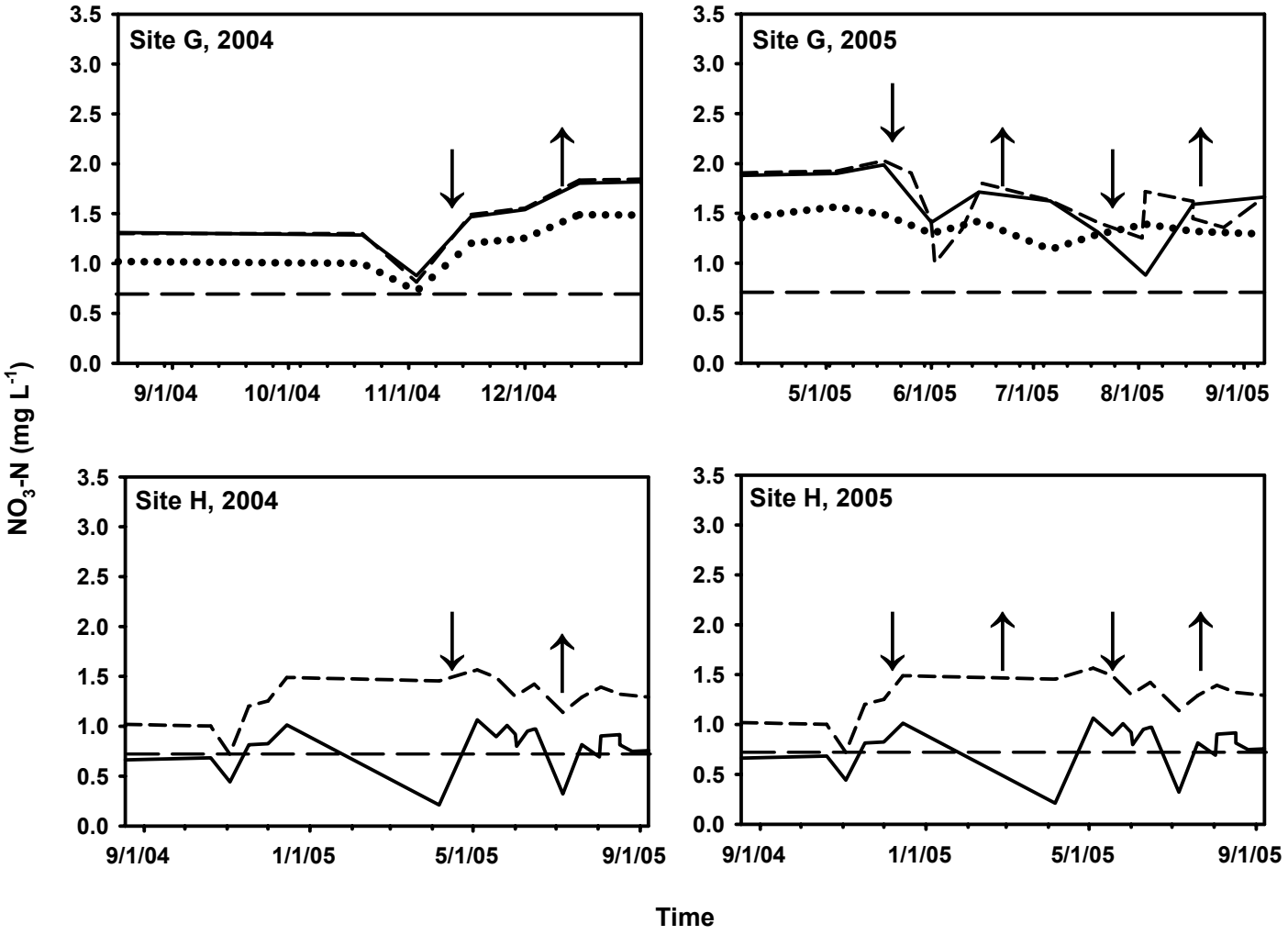

Figure 3.10. Background NO_3-N concentrations for Rose Creek, lower reaches, from September 2004 to September 2005. EPA NO_3-N concentration guideline (0.69 mg L^{-1}) denoted by long-dashed line. Solid lines are concentrations just upstream from periphytometer locations. Dark-dotted lines are concentrations at sampling locations further upstream. Arrows pointing downward denote dates periphytometers were deployed and arrows pointing upward denote dates periphytometers were removed.

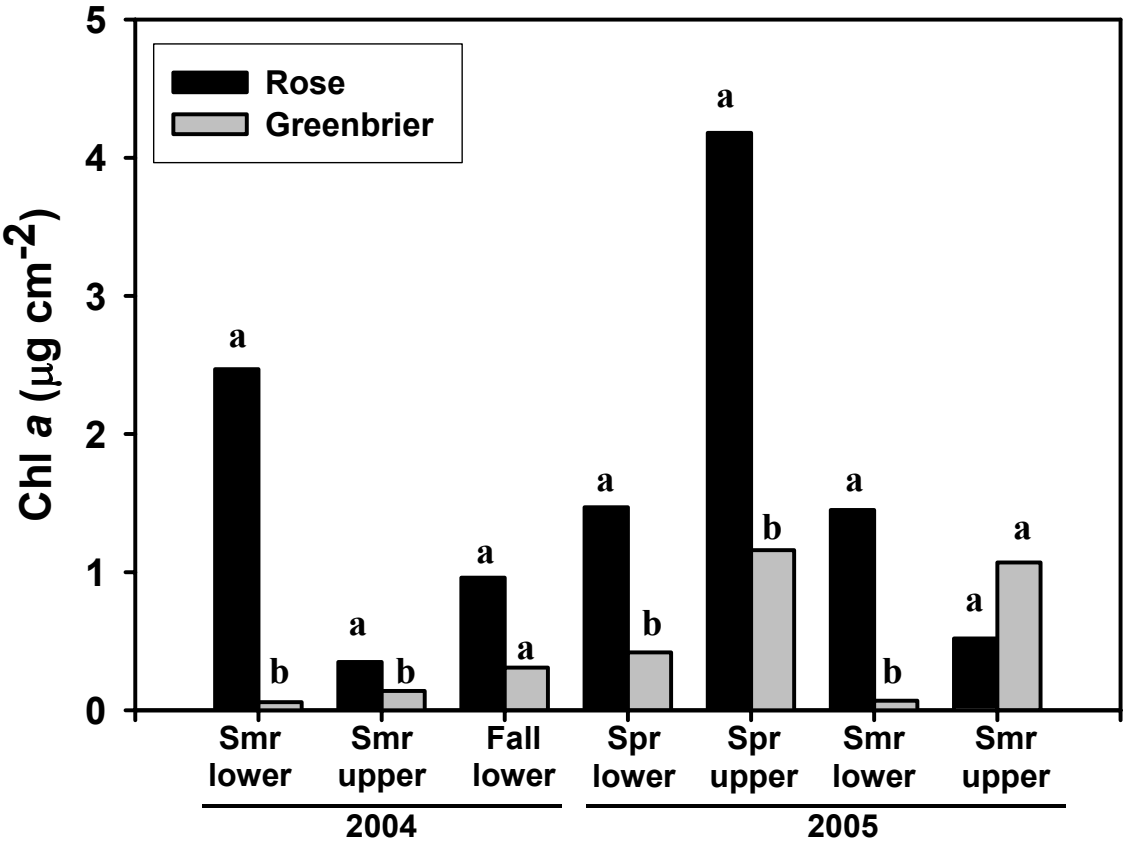

Figure 3.11. Comparison of chl *a* concentrations across bottles and vials and treatments between streams in the upper and lower reaches of the Rose and Greenbrier Creeks. "Smr" is summer and "Spr" is spring. Each set of bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$.

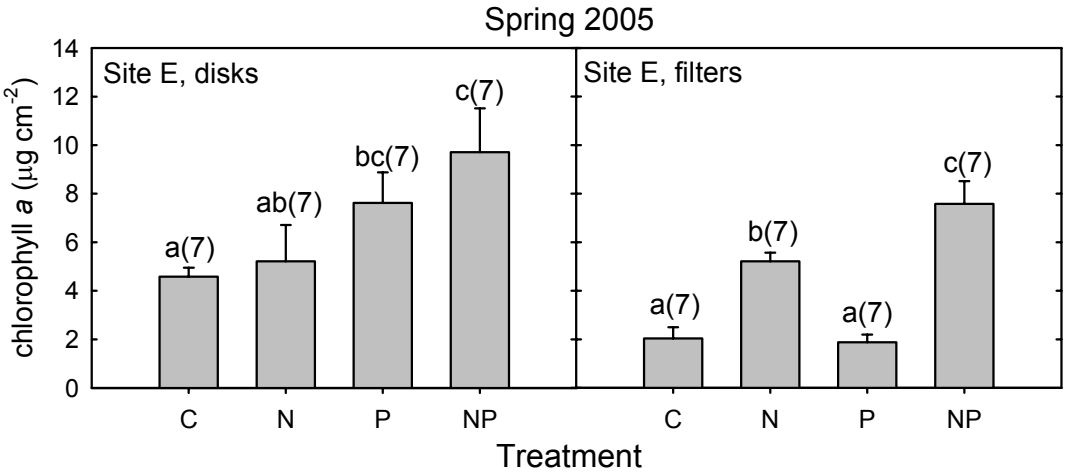

Figure 3.12. Disk and filter chl *a* concentrations for Rose Creek sampling site E, upper reaches, spring 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Numbers in parentheses are sample size.

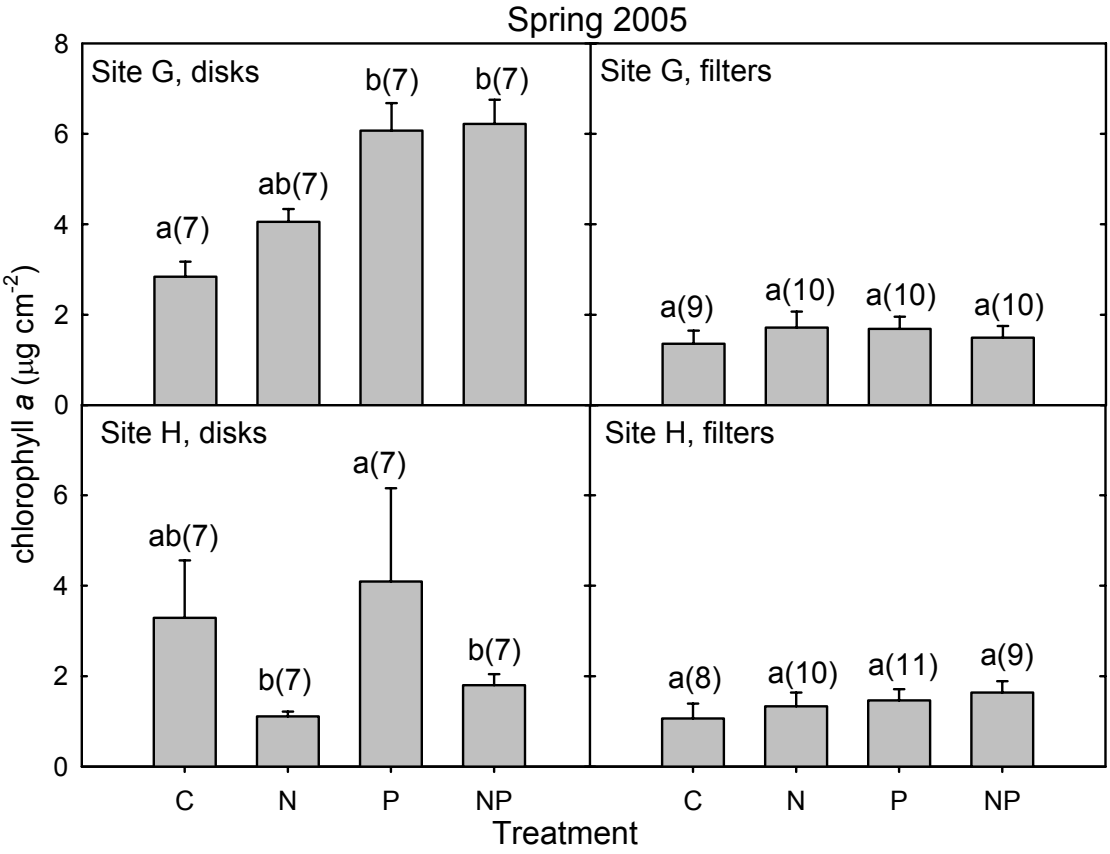

Figure 3.13. Disk and filter chl *a* concentrations for Rose Creek sampling sites, lower reaches, spring 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Numbers in parentheses are sample size.

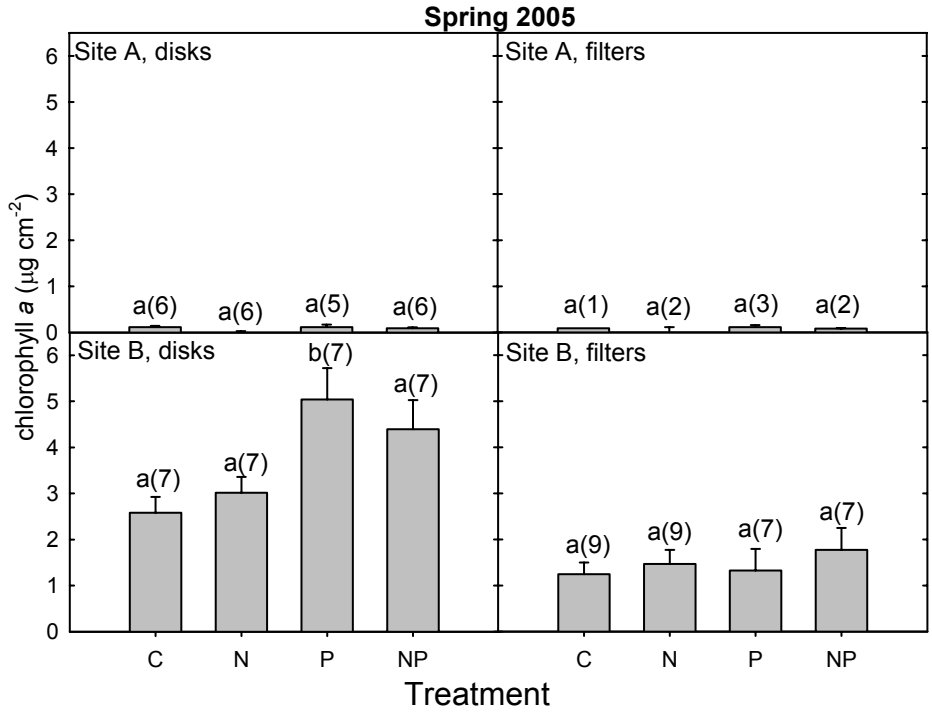

Figure 3.14. Disk and filter chl *a* concentrations for Greenbrier Creek sampling site, upper reaches, spring 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Sample size is in parentheses.

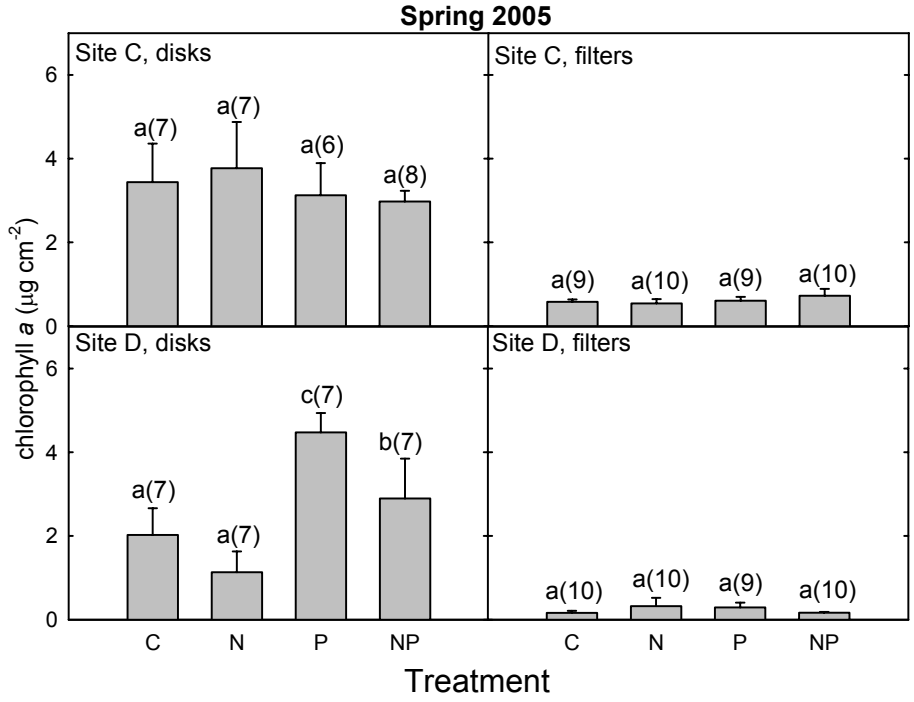

Figure 3.15. Disk and filter chl *a* concentrations for Greenbrier Creek sampling site, lower reaches, spring 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Sample size is in parentheses.

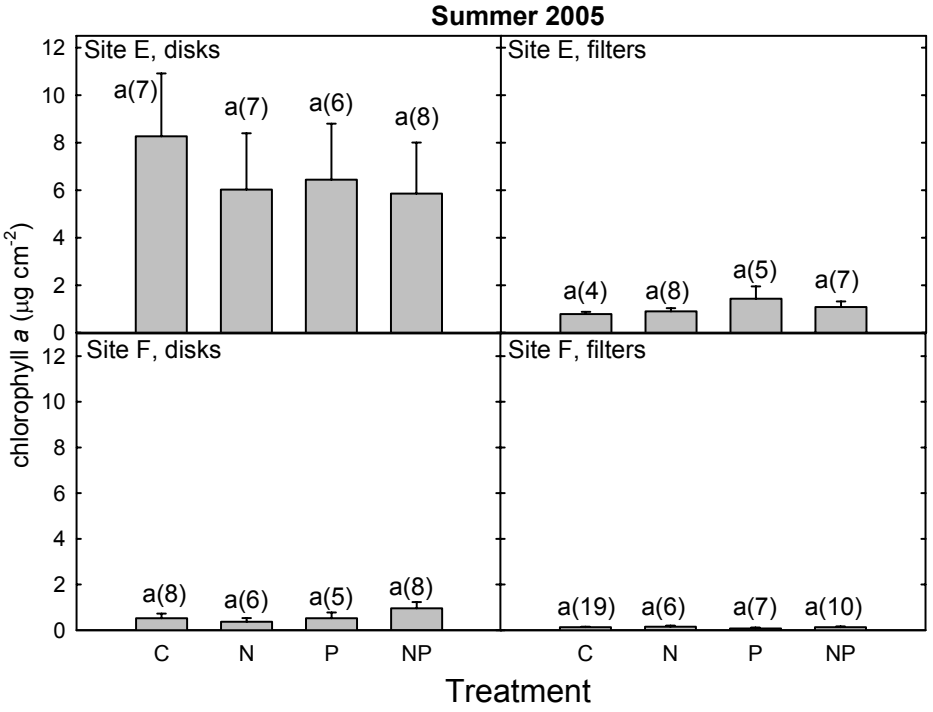

Figure 3.16. Disk and filter chl *a* concentrations for Rose Creek sampling site, upper reaches, summer 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Sample size is in parentheses.

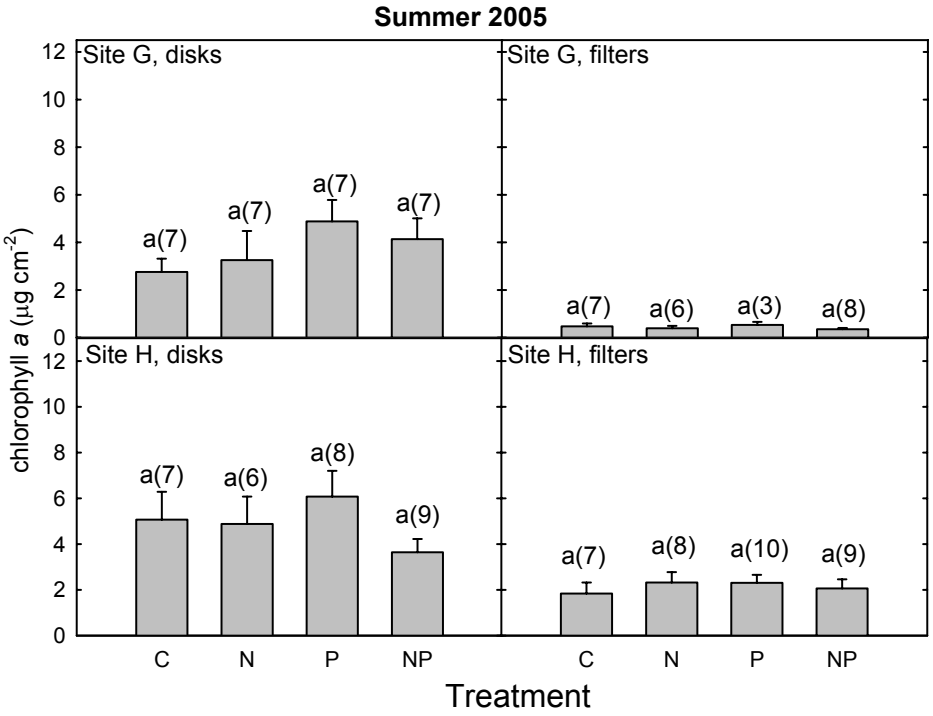

Figure 3.17. Disk and filter chl *a* concentrations for Rose Creek sampling sites, lower reaches, summer 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Sample size is in parentheses.

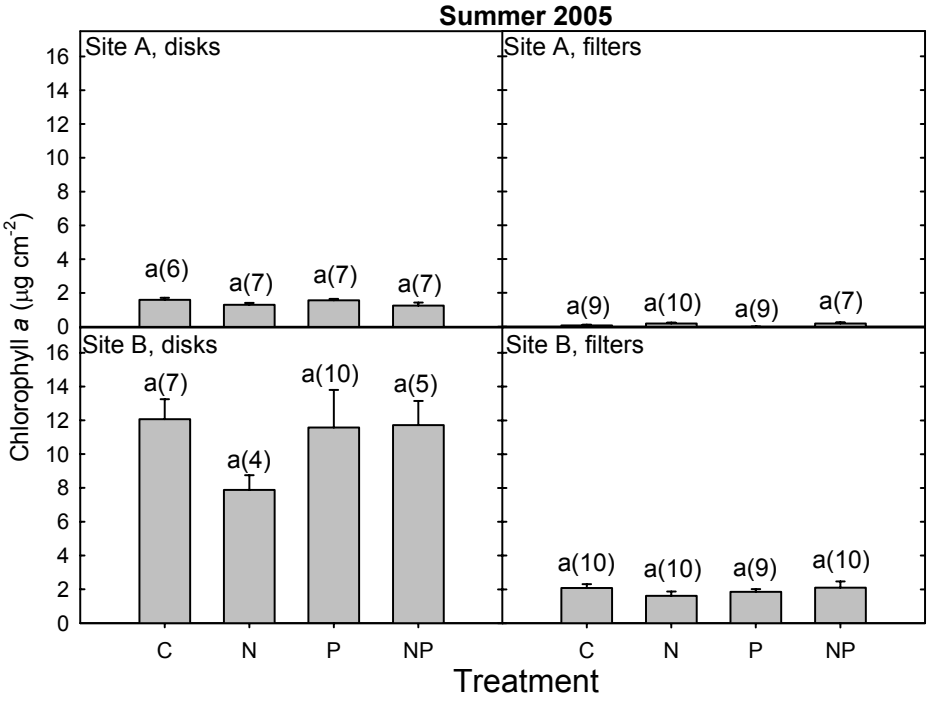

Figure 3.18. Disk and filter chl *a* concentrations for Greenbrier Creek sampling sites, upper reaches, summer 2005. Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Sample size is in parentheses.

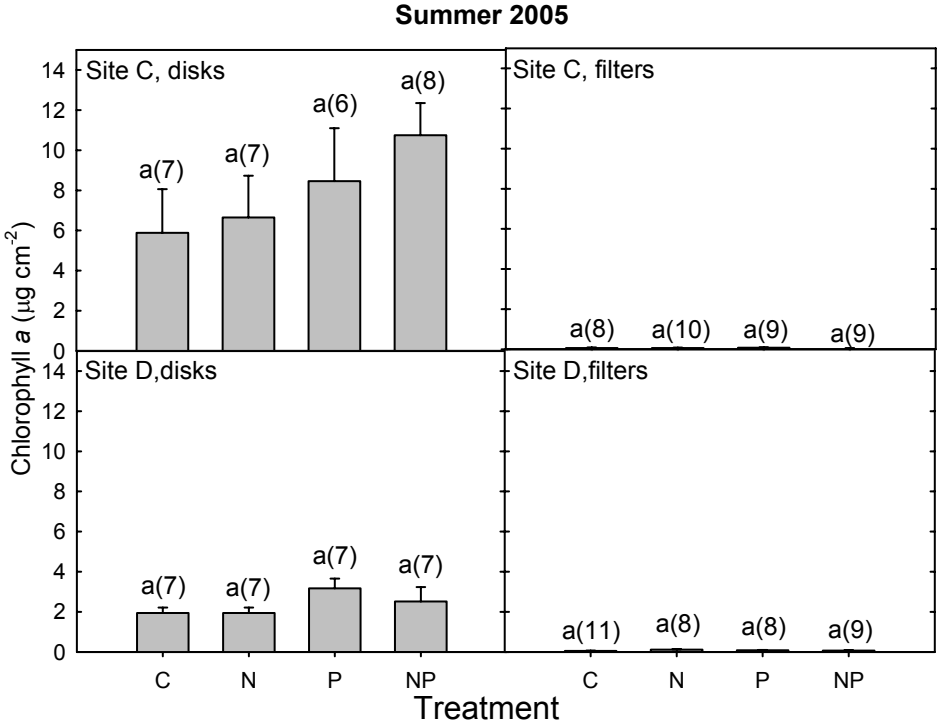

Figure 3.19. Disk and filter chl *a* concentrations for Greenbrier Creek sampling sites, lower reaches, summer 2005. (Within sites, bars with same letter are not significantly different according to Fisher's LSD at $p<0.05$. Sample size is in parentheses.

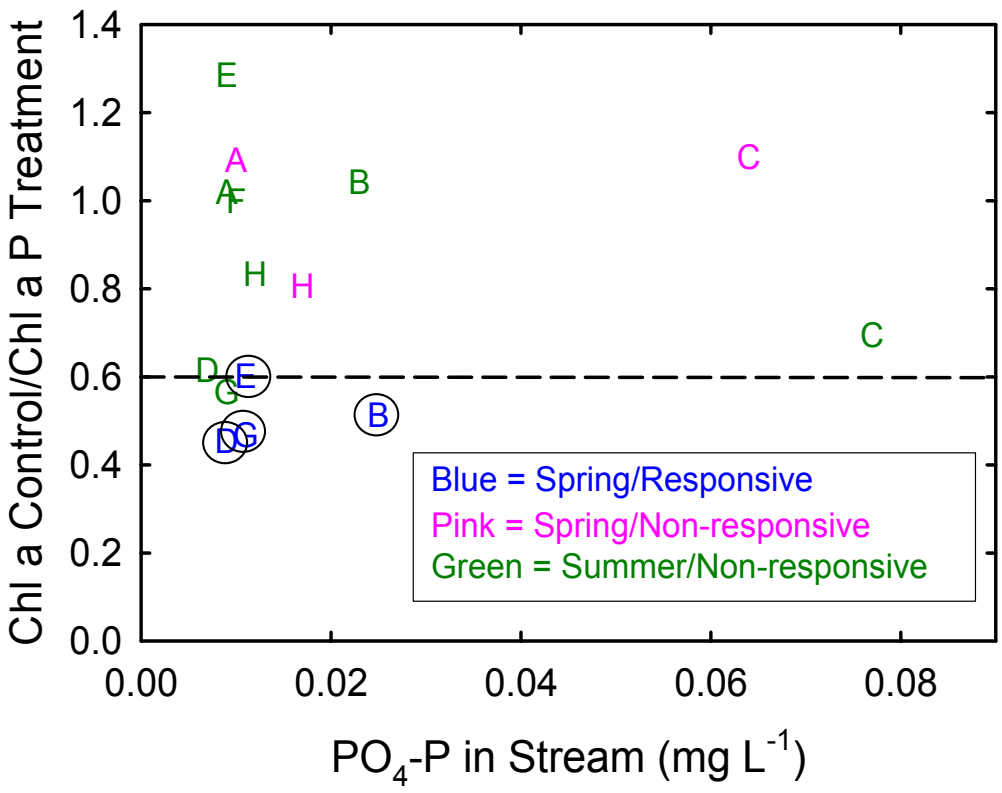

Figure 3.20. Stream Phosphorus Trophic Status as a function of background stream PO_4-P concentrations for periphytometer deployments in Spring and Summer 2005 at eight stream sites in two Georgia Piedmont watersheds. Circled sites are those that responded to P-treatment in spring 2005.

CHAPTER 4

CONCLUSIONS

Preliminary Testing of Nutrient-Diffusing Substrates

Nutrient-diffusing bottles exposed to an experimental creek experienced rapid loss of nutrients, but final concentrations of NO_3-N and PO_4-P after 24 d of exposure were above the nutrient criteria for ecoregion IX (0.036 mg P L^{-1} and 0.69 mg N L^{-1}). These results suggest that nutrient-diffusing bottles should provide sufficiently high concentrations to stimulate algal growth in nutrient-deficient streams. A maximum of 15% of the initial PO_4-P in nutrient-diffusing agar was lost during 5 d of exposure in an experimental creek, and no evidence of complete depletion of the PO_4-P in the upper 1 cm of agar was observed. Based on these results, concentrations of 15,500 mg PO_4-P L^{-1} and 7000 mg NO_3-N L^{-1} were selected for later field studies.

When both filters and disks were tested as a substrate for periphyton growth, disks had greater chl *a* concentrations in most comparisons. This may be a result of the orientation of the disk to irradiance. The disks received more irradiance than the filters, which were oriented perpendicularly to the surface of the water and parallel to irradiance. Therefore, the filters may not have received enough irradiance to support the same periphyton community structure as the disks. This is further supported by regression analysis for filters which indicated an inverse relationship between PAR and chl *a* concentration. In conclusion, disks are better for measuring

periphyton growth than filters because they have more surface area for growth, they consistently produced greater chl *a* concentrations, and are more resistant to loss in Georgia Piedmont streams.

Determination of Limiting Nutrient for Periphyton Growth in Piedmont Streams

Using the nutrient-diffusing bottles, only one site (site E) out of seven was limited by N in spring 2005. The NO_3-N concentration in the stream was 2.9 mg L^{-1}. The lack of P response with the filters could have been caused by the P flux through the filter which was about 100-fold less than the flux through the disks. In contrast, P-limitation was indicated for four sites (sites E, G, B, and D) out of seven in spring 2005 when using nutrient-diffusing agar vials. The PO_4-P concentration in the responsive sites ranged from 0.01 to 0.03 mg P L^{-1}. In the summer, the same sites did not show a response to P enrichment. Possible explanations for the different response to treatments in the spring and summer may be different amounts of PAR received by the growing surface and possible seasonal changes in periphyton community structure.

The Phosphorus Trophic Status, calculated as the ratio of chl *a* concentration on the control over the chl *a* concentration on the P-treated vials, showed that P-limited sites in spring 2005 had PTS values at or below 0.6. In the summer 2005, none of the sites responded to P treatment and PTS values were at or above 0.6. This suggests that it may be possible to identify a threshold PTS value below which a P response, as indicated by chl *a*, would be expected. If such a PTS threshold is identified, it would be useful to explore the possibility of estimating PTS for a stream from environmental variables. In this study, no strong relationship was found between

PTS and stream PO_4-P concentration, indicating that additional work should be conducted to explore other variables that may be strongly related to PTS.