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Abstract

Availability of nutrients, particularly nitrogen (N) and phosphorus (P), is a key driver of microbial growth and metabolism in freshwater ecosystems. Microbial biofilms composed of both autotrophs and heterotrophs play crucial roles in freshwater food webs. These stream communities may be limited by N, P, or both N and P. This project examined nutrient limitation of biofilm communities in two small, freshwater streams in the Catawba river basin within the Catawba Nation in South Carolina. Stream conditions were monitored and nutrient concentrations for ambient ammonium and phosphate were monitored. We quantified nutrient limitation, community respiration, gross primary production, and ash-free dry mass of biofilms on two substrate types, selecting for autotrophs or heterotrophs, using nutrient diffusing substrates (NDS). NDS allow for slow, constant nutrient amendments for autotrophic and heterotrophic communities without affecting the rest of the ecosystem. Ambient nutrient concentrations in both streams were relatively low. Nevertheless, for all parameters except community respiration in one stream, microbial biofilms did not appear to be nutrient limited. In one stream, community respiration on substrates selecting for heterotrophs suggested those communities may be nutrient limited. Understanding how nutrients impact microorganism growth and metabolism in these streams is important for managing these resources and managing anthropogenic inputs in the surrounding watershed.

Introduction

Nutrients are needed for the growth of organisms and are vital to ecosystem processes. Two of the most important nutrients in freshwater ecosystems are nitrogen (N) and phosphorus (P) (Elser et al. 1990). The availability of essential nutrients is a driver for most ecosystem processes, such as productivity and decomposition (Meyer et al. 1998).

Biofilms in freshwater streams, comprised largely of algae, cyanobacteria, heterotrophic bacteria, and fungi, play a critical role in a variety of stream ecosystem functions (Tank et al. 2003). Heterotrophs prefer to grow on organic substrate while autotrophs prefer to grow on inorganic substrate (Tank and Dodds 2003). Autotrophs and heterotrophs in these biofilm communities may also be limited by different nutrients (Johnson et al. 2009).

Single species algal cultures are often limited by a single nutrient, but multispecies algal communities could be limited by multiple nutrients since different species of algae can have different nutrient requirements (Francoeur 2001). Biofilm communities are highly likely to be multispecies and not monocultures (Francoeur 2001). It has also been shown that freshwater benthic stream periphyton are likely to be colimited by both N and P (Elser et al. 2007). A metanalysis of stream benthic algal communities found colimitation to be more common than limitation by N or P alone (Francoeur 2001).

The objective of this study was to determine nutrient limitation of biofilm communities in two small streams in the South Carolina Piedmont. We predicted that the streams in the Catawba Nation Reservation would be limited by both N and P. Nutrient limitation is frequently assessed using nutrient diffusing substrates (NDS), which allow small amounts of nutrients to enter a system and promote autotrophic or heterotrophic growth. NDS were assessed for metabolism and ash-free dry mass (AFDM).

Methods

Site selection We conducted this study in two small streams (CAT05 and CAT15) on the Catawba Nation Reservation in Rock Hill, SC. This area is in the ecoregion of the Carolina Slate Belt, classified by volcanic rocks in the form of slate and granites (Griffith et al. 2002).

Nutrient Diffusing Substrates NDS were constructed using 30 mL plastic containers filled with a 2% agar solution topped with either fritted glass (targeting autotrophic colonization) or cellulose sponge discs (targeting heterotrophic colonization). Agar solutions were amended with the following treatments: control (no nutrients added) N addition, P addition, and N + P addition (n = 5 per treatment per substrate). Nitrogen additions were created using KNO_3. Phosphorus additions were created using KH_2PO_4.

NDS Deployment and Collection NDS were incubated in each stream for 19 days in March 2020 prior to full canopy leaf out (n = 40 per stream). During deployment and at weekly intervals, physical and chemical parameters were measured at each stream, including current velocity, dissolved oxygen, conductivity, and temperature. HOBO Dataloggers recorded light and temperature in 1-hour increments. Water samples were also collected to quantify phosphate (APHA 2012) and ammonium (Holmes et al. 1999, Taylor et al. 2007).

Metabolism NDS discs were incubated with stream water in 50 mL centrifuge tubes in the light for three hours followed by three hours in the dark. Initial and final dissolved oxygen concentrations (mg/L) were measured for each incubation. The difference between these two measurements in the light incubation represents net ecosystem production (NEP) and in the dark incubation represents community respiration (CR). These two values together represent gross primary production (GPP).

Ash Free Dry Mass After the metabolism incubations, the discs were placed in a preweighed aluminum pan and dried in a drying oven at 50 °C. After drying, the discs were cooled and then weighed. Then the discs were combusted in a muffle furnace at 500 °C for 2 hours, cooled, and weighed again. AFDM is the difference between the two masses.

Statistical Analyses To test for treatment effects on GPP, CR, and AFDM, a one-way analysis of variance (ANOVA) was used. Data for ANOVAs were square root transformed to meet assumptions of normality and homoscedascity. Transformations of CAT05 autotrophic AFDM did not result in data that passed these tests, so a Kruskal-Wallis test was used to test for treatment effects on those data.

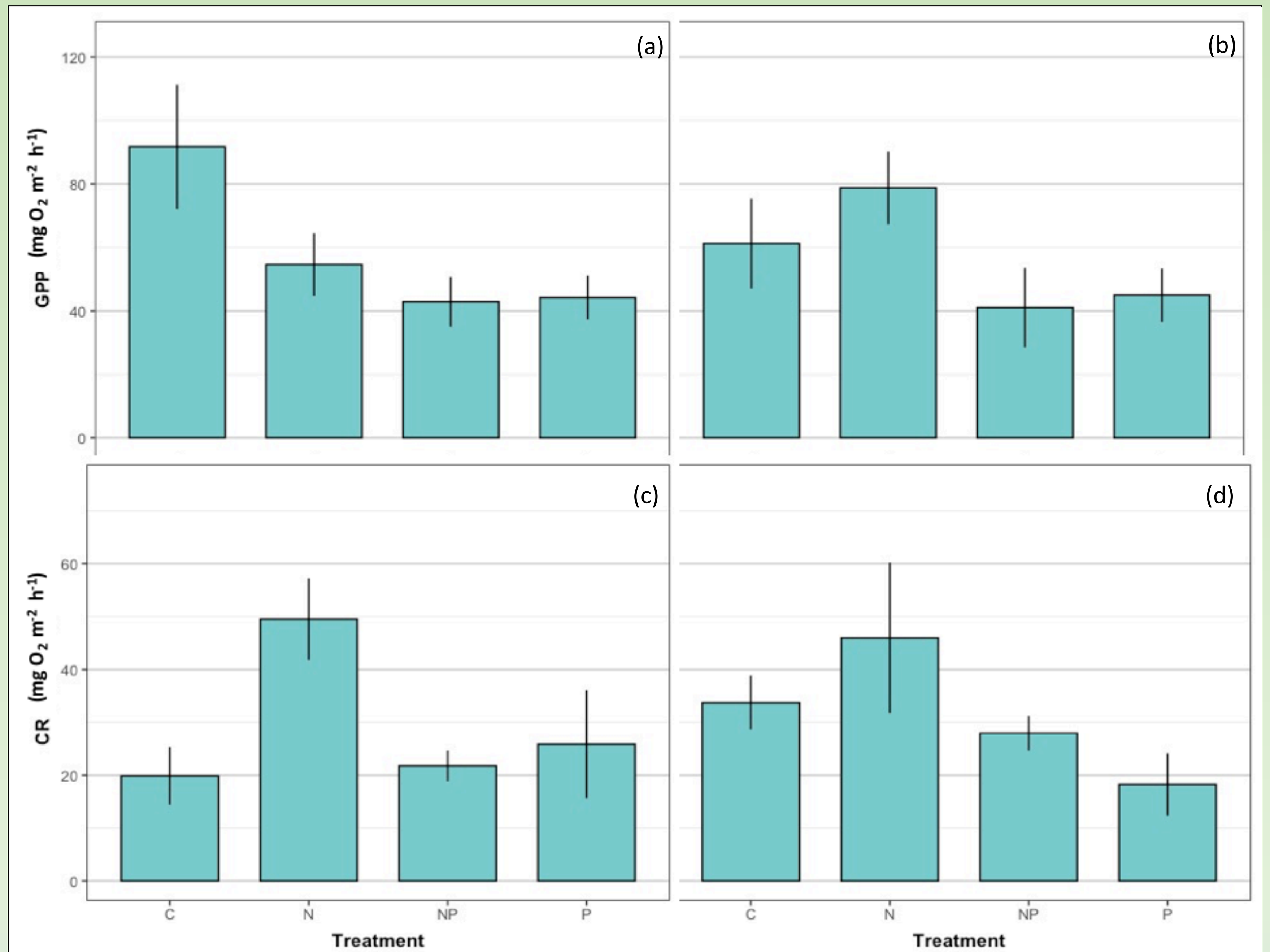

Figure 1. Metabolism Data for CAT05 and CAT15. GPP (gross primary production) of autotrophic communities in (a) CAT05 and (b) CAT15. CR (community respiration) of heterotrophic communities in (c) CAT05 and (d) CAT15. Error bars represent ± 1 SE.

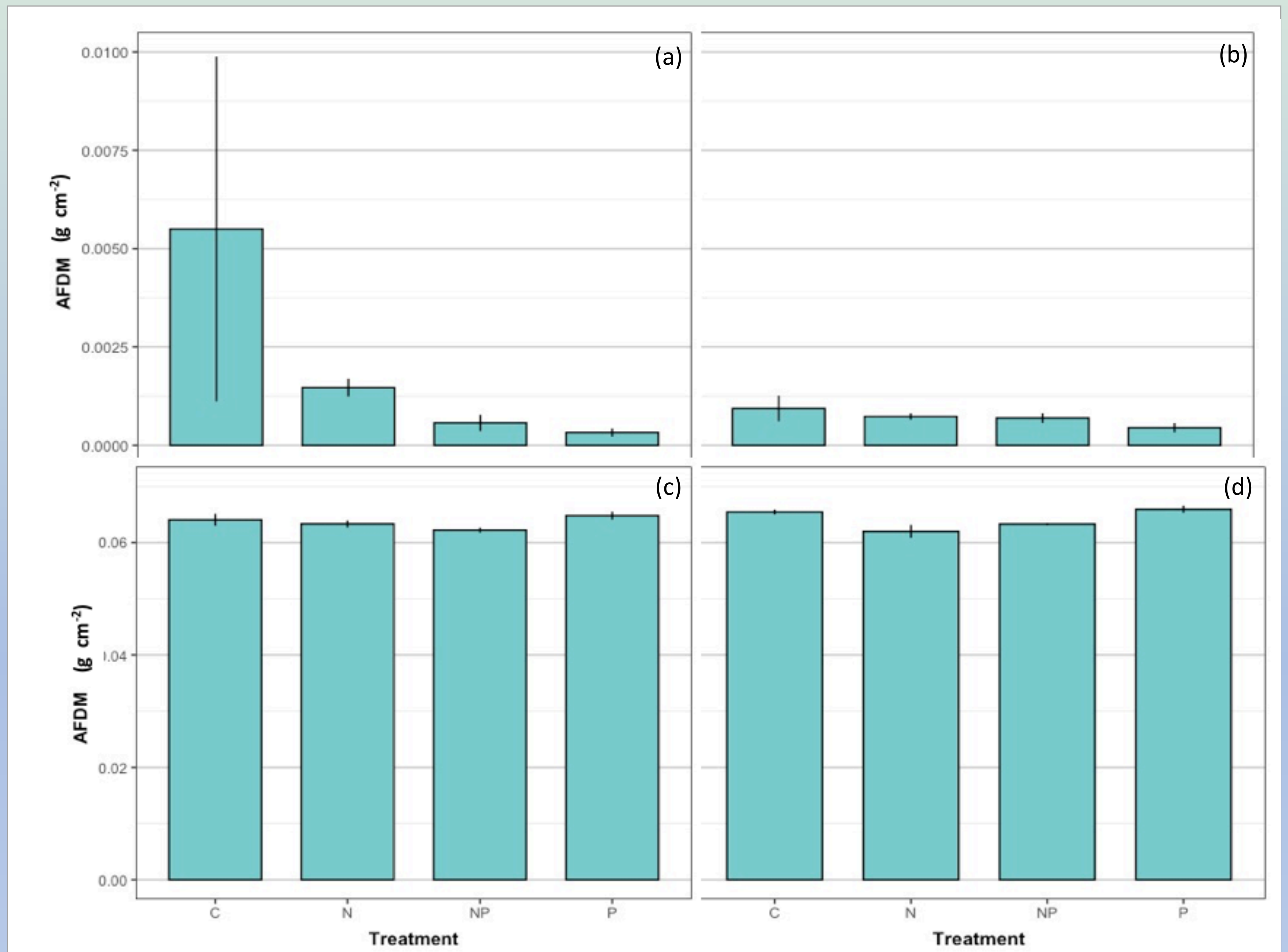

Figure 2. Ash Free Dry Mass Data for CAT05 and CAT15. AFDM of autotrophic communities for (a) CAT05 and (b) CAT15. AFDM of heterotrophic communities for (c) CAT05 and (d) CAT15. Error bars represent ± 1 SE.

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Results

- Light for CAT05 peaked at 21,000 lux and at 31,500 lux for CAT15.
- Temperature ranged between 12.4 °C and 23.2 °C for CAT05. CAT15 ranged between 11.0 °C and 23.5 °C.
- Ambient nutrient concentrations (phosphate and ammonium) were generally low in both streams.
- GPP in CAT05 decreased in response to nutrient amendments ($F_{3,16}$, p = 0.04; Figure 1a). The difference between the control treatment and both P and NP treatments was marginally significant (Tukey's HSD, p = 0.07 and 0.05, respectively).
- There was no significant difference in GPP in CAT05 or in CR in either stream ($F_{3,16}$, p > 0.05; Figure 1b-d), though nitrogen treatments were higher than the control and the other nutrient treatments.
- AFDM of autotrophic communities in CAT05 were significantly different (H_3 = 11, p = 0.01, Fig. 2a), though this difference was likely driven by one high value in the control treatment.
- AFDM pf heterotrophic communities in CAT15 were also significantly different ($F_{3,16}$, p = 0.002; Figure 2d). Neither autotrophic AFDM in CAT15 nor heterotrophic AFDM in CAT05 were significantly different ($F_{3,16}$, p > 0.05; Figure 2b, c).

Discussion

Heterotrophic communities in both streams showed a slight positive response to nitrogen additions; however, the differences among treatments were not significant. This is consistent with previous findings that heterotrophs are more responsive to nutrient additions than autotrophs (Johnson et al. 2009).

Autotrophic communities in one stream unexpectedly had significant inhibition of GPP in treatments containing phosphorus. Though rare, this has been observed in other experiments (Francouer 2001).

Ash free dry mass did not show much response to nutrient additions for either stream or community except CAT05 autotrophic communities. However, there was a large amount of ash free dry mass on a single control sample, meaning there may have been excess growth in the particular location where that replicate was placed (all NDS were attached in random order to plastic L-bars on the stream bottom). Although GPP was also higher for autotrophic controls, there was no correlation with GPP and AFDM.

Future analysis may look at how characteristics of the stream, such as season or water velocity, affect nutrient limitation. It would also be informative to compare the effects of factors such as grazers and other disturbances. Understanding how multiple nutrients differentially affect communities in aquatic systems is an important step in managing aquatic resources.

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