USING MULTIPLE LENSES TO INVESTIGATE TROPHIC INTERACTIONS AND RESPONSES TO ECOLOGICAL STRESSORS IN LOTIC ECOSYSTEMS

by

JAMES LINDLEY WOOD

(Under the Direction of MARY FREEMAN)

ABSTRACT

Freshwater resources around the world are increasingly impacted by human activity. Conversion of forested land into agriculture land and increasing urbanization in watersheds has been correlated with increased nutrient concentrations in surface water and alteration to the natural flow regime. These modifications can decrease resource availability to benthic organisms by decreasing standing stocks of basal resources and increasing carbon export from the system, or stimulate undesirable algal accrual. Because nutrient enrichment and flow alteration can have deleterious impacts on freshwater biodiversity and ecosystem services, we investigated how nutrient enrichment and flow affected basal resources in mid-sized streams and rivers in the eastern U.S. We investigated changes in basal resource structure and function at multiple scales (a few meters to 100s of kilometers) by using a variety of short term (24 hours) and long-term (up to 2.5 months) experiments. To test the effects of increased nutrient availability on autotrophic and heterotrophic biofilms, we deployed nutrient diffusing substrates into 15 streams in the Little Tennessee River watershed in the Southern Appalachian Mountains. We found that gross primary production was phosphorus limited on epilithic surfaces but that algal biomass was co-limited by nutrients. We also found that the effects of consumers and scour may obscure the effects of nutrients on labile carbon sources. To test the effects of flow alteration on basal resources, we setup a series of experiments to explore how water velocity and herbivory interacted to regulate biomass accrual of the widespread riverine macrophyte *Podostemum ceratophyllum*. We found that water velocity reduced consumer access to the plant, and that low water velocity conditions facilitate the transfer of stored biomass into the food web. Lastly, we analyzed *Podostemum* from rivers between Georgia and Maine for nutrient content (C, N, P), metals (Cd, Na, Zn) and isotopically enriched nitrogen ($\delta^{15}N$). We found that land use was reflected in the elemental composition of *Podostemum,* which has implications for nutrient storage and flux, and presents a link by which trace metals from urbanized landscapes can move into food webs at potentially toxic levels. Collectively, these study provide new insight into the various ways in which anthropogenic activity impact basal resource in freshwater ecosystems.

INDEX WORDS: Podostemaceae, ecology, nutrient, flow, velocity, Podostemum, ceratophyllum, macrophyte, herbivory, land use, metals, trophic, carbon, nitrogen, phosphorus, microbial respiration, gross primary production, nutrient diffusing substrate

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DEDICATION

This dissertation is dedicated to the many wonderful dogs in my life, who were always ready for an adventure and were just as excited as I was to go outside and investigate the doings of the world. I would also like to dedicate this dissertation to my father, Roger, for introducing me to rivers, canoeing and kayaking, and to my mother, Theresa, for always encouraging my interests in plants, animals and the many tiny things in the world.

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CHAPTER 1

INTRODUCTION

Ecosystem function and structure are controlled by resource availability (Tank and Dodds, 2003; Johnson et al., 2009) and abiotic stressors (Poff et al., 2009; Bernot et al., 2010), and in turn influence the elemental flux of materials from one pool to another (Dixon et al., 1994; Rosemond et al., 2015). These fluxes and transformations of materials are drivers of species interactions (Davis et al., 2010a), influence habitat complexity (Lee and Hershey, 2000), primary production (Elser et al., 2007) and secondary production (Davis et al., 2010b). In order to better understand the connections between interacting components, investigators must utilize multiple lenses to explore the ecological linkages and disconnects (Poole, 2002) on multiple spatial scales.

River systems present unique challenges to researchers, due to autocorrelation between upstream and downstream river segments (Douglas et al., 2000), hydrological and ecological characteristics that change with stochastic events (Strange et al., 1993; Sabo and Post, 2008), differences in geologic influence (Montgomery, 1999), and anthropogenic impacts that have both proximate and distant influences (Walsh et al., 2005; Wenger et al., 2009). However, the need to better manage freshwater for human health (Conley et al., 2009; Paerl et al., 2011), ecosystem services (Palmer and Febria, 2012; Dodds et al., 2013) and the conservation of biodiversity (Freeman and Freeman, 1994; Strayer and Dudgeon, 2010) is pressing. Furthermore, the need to manage the functional and structural integrity of natural resources, especially freshwaters (Vörösmarty et al., 2000), is heightened by the amplifying signal of climate change

(McMichael et al., 2006) and a growing global population (Cohen, 1995). My research has examined carbon balance, trophic interactions and the effects of land use on aquatic ecosystems basal resources, because basal resources are a key resource in lotic systems that are susceptible to influences from anthropogenic activity and changes in basal resources can influence ecosystem function and food web structure, including higher-level consumers.

Chapter 1 examines the influence of increased nutrient availability on 2nd to 3rd order streams in a large watershed in the southern Appalachian Mountains. By using nutrient diffusing substrate and consumer exclosure experiments. I tested for nutrient limitation of gross primary production, community respiration and algal biomass on both inorganic and organic substrates to assess similarities and differences of biofilm response to nutrient enrichment.

In Chapter 2, I explore the current state of knowledge concerning the ecology of the eastern North American macrophyte *Podostemum ceratophyllum* Michx. I propose that *P. ceratophyllum,* henceforth referred to as *Podostemum*, is a foundation species of rivers throughout the piedmont and montane regions because of the plant's substantial positive influence on macroinvertebrate biomass, organic matter dynamics, and habitat complexity. After reviewing the available literature on the plant, I hypothesize several interactions between plant biomass, water velocity, herbivory, and land-use associated anthropogenic water pollution. I conclude this chapter with predictions of changes in ecological structure and function in rivers where *Podostemum* biomass is removed from the system.

Chapter 3 investigates the influence of water velocity on herbivory pressure of *Podostemum* by quantifying changes in plant biomass and stem length in response to manipulations of water velocity and consumer access. I hypothesize that herbivory plays a significant role in limiting *Podostemum* biomass accrual and also that water velocity interacts with herbivory pressure, by which herbivory pressure in high-velocity habitats is substantially reduced compared to low-velocity habitats. Lastly, I hypothesize that herbivory, not water velocity, is the primary influence defining the species realized niche. These results provide substantial evidence to support the hypotheses and this study provides insight into how management decisions concerning water extraction and release, and stochastic events such as drought, can influence basal resource accrual in eastern U.S. rivers.

Chapter 4 investigates the nutrient content and potential for metal uptake by *Podostemum.* Plant samples were collected from 34 rivers in 11 eastern states and I examine changes in the concentrations of $\delta^{15}N$ (an isotope of nitrogen), carbon (C), nitrogen (N), phosphorus (P), cadmium, sodium, and zinc, and molar ratios of C, N and P in relation to watershed land use. Results support the conclusion that the plant's elemental composition is reflective of land use, specifically loss of forest cover and various forms of urban development. These results help elucidate how land use within the watershed of a river can influence the quality and toxicity of basal resources, specifically *Podostemum,* in the eastern U.S.

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CHAPTER 2

HETEROTROPHIC AND AUTOTROPHIC RESPONSES TO NUTRIENT ADDITION IN SOUTHERN APPALACHIAN STREAMS[1]

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[1] Wood, J. L., Amy D. Rosemond, John S. Kominoski, and S. J. Wenger. 2017. To be submitted to Hydrobiologia.

Abstract

Worldwide nutrient enrichment of streams and rivers is changing their trophic structure and ability to store and release carbon. Enrichment is also expected to continue with increasing global population growth. However, predicting responses to additional enrichment in lotic systems already enriched with N and P remains challenging, in particular because thresholds of limitation of can differ between heterotrophic and autotrophic communities. We sought to compare nutrient limitation between autotrophic and heterotrophic communities in the southern Appalachian Mountains to better understand landscape-scale trends in nutrient limitation. We used nutrient diffusing substrates (NDS) and consumer exclosures to examine nitrogen (N) and phosphorus (P) limitation of gross primary production (GPP), community respiration (CR), and algal biomass on organic (labile cellulose sponge) and inorganic substrates (fritted glass) in 15, 2^{nd} – 3^{rd} order streams. We assessed changes in metabolism (GPP, CR) and algal biomass (inorganic substrates only) in response to N and P enrichment after 21 days across a range of surface water dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) concentrations. In the NDS experiment, we found widespread P limitation of GPP on inorganic substrates and co-nutrient limitation (N+P) of algal biomass. On organic substrates, neither CR nor GPP were found to be nutrient limited. We found that streams were highly variable in their response to nutrients. Canopy cover was an important variable, and reduced the GPP response to nutrients, specifically in the P treatment. Community respiration increased with increasing N and canopy cover, possibly indicating changes in community composition and trophic structure. We found only weak relationships between algal biomass and canopy, but saw evidence that canopy

cover over 80% may limit algal accrual. Significant relationships between water chemistry and community respiration were observed in the consumer exclosure experiment, where CR on organic substrates was correlated with stream water DIN and SRP. These results indicate continued nutrient enrichment of freshwater can have large influences on metabolism by epilithic, autotrophic biofilm communities.

Introduction

There is an urgent need to improve our understanding of how freshwater systems are responding to increasing nutrient enrichment, as there is growing evidence that nutrient enrichment is harmful to human health and diminishes ecosystem services (Paerl et al. 2011; Paerl et al. 2016). Structural metrics, such as algal biomass (Elser et al. 2007; Francoeur 2001), are most frequently used to assess nutrient limitation in freshwater streams, rivers and lakes. However, structural responses to nutrient enrichment can vary considerably among ecoregions due to differences in light (Lowe et al. 1986; Rosemond 1994; Rosemond et al. 1993), nutrient availability, catchment geomorphology (Montgomery 1999), and climate (Bernot et al. 2010; Johnson et al. 2009). For example, thresholds of nitrogen (N) limitation vary widely, occurring between 150 µg L^{-1} Total N (Dodds et al. 2002) to just over 1350 µg L^{-1} Dissolved Inorganic Nitrogen (DIN) (Keck and Lepori 2012). Similarly, phosphorus (P) limitation may be more important than N limitation in freshwater systems because of atmospheric N fixation by some algal species (Schindler et al. 2008). Thus, dual nutrient reduction strategies appear necessary to prevent toxic algal blooms (Paerl et al. 2011) and hypoxic zones in downstream lakes and marine systems (Dodds and Welch 2000; Howarth et al. 2011; Paerl et al. 2016). However, there is little information available about how larger streams (2-3rd order) that are already modified by anthropogenic actives and moderately enriched with nutrients will respond to additional nutrient enrichment.

Because most nutrient enrichment studies have been conducted in small shaded streams using structural metrics, the current state of knowledge concerning functional responses to nutrient enrichment, such as Gross Primary Production (GPP) and

Community Respiration (CR), in larger streams with increased light availability is lacking (Palmer and Febria 2012). Nutrient enrichment is reported to increase CR rate and carbon loss from headwater streams (Gulis et al. 2004; Johnson et al. 2009; Kominoski et al. 2015; Rosemond et al. 2015; Woodward et al. 2012) because of increased microbial and invertebrate consumer activity. The loss of carbon storage capacity in streams reduces basal resources for aquatic consumers and influences the rate at which carbon is transported downstream. However, increases in algal biomass resulting from increased nutrient availability can increase carbon storage and may counteract the effects of increased carbon export. These changes in carbon loss and storage impact higher trophic levels and predator-prey dynamics (Davis et al. 2010a), and may affect downstream ecosystems and services provided by streams.

Despite the growing number of studies assessing nutrient limitation and carbon balance in low nutrient “pristine” headwater streams, there are still deficiencies in our understanding of the ways in which nutrient enrichment interacts with benthic carbon stocks and light to influence stream metabolism. Significant differences in the responses by autotrophic and heterotrophic communities to enrichment have previously been reported (Hoellein et al. 2010; Johnson et al. 2009; Mosisch et al. 2001; Tank and Dodds 2003). However, substantial variation in nutrient status of streams across the landscape warrants further investigation into biofilm responses to nutrient enrichment in streams already experiencing increased nutrient concentrations. Furthermore, assessing landscape scale responses to enrichment is rare but can provide valuable insight into watershed scale patterns of carbon loss and storage, and may elucidate mechanistic relationships between resource availability (nutrients and light) and carbon balance. Additionally, assessments of interactions between light, algal biomass, microbial activity, and consumer contributions to carbon loss may provide important insight into management strategies to protect freshwater resources and downstream ecosystems.

We investigated heterotrophic (CR) and autotrophic-pathway (GPP and biomass accrual) responses to increased nutrient availability in 2nd-3rd order streams across a wide range of light availability and background nutrient concentrations. We tested the following hypotheses: 1) that across the landscape, algal biomass (chl *a*), GPP, and CR are co-limited by N and P; 2) that nutrient limitation is predictable by background steam water nutrient concentrations and light availability; 3) that CR increases faster than GPP with nutrient addition; and 4) that nutrients effects on microbial respiration rates are in part controlled by consumers.

Methods

Study site

To test autotrophic and heterotrophic responses to increased nutrient availability we conducted two landscape-scale experiments in the Little Tennessee River watershed in North Georgia and Western North Carolina (Fig. 1). Streams were 2-3rd order, and land use varied from forested, to agricultural, to ex-urban and urban.

Experiment 1: Nutrient Diffusing Substrates

Nutrient limitation and carbon balance were investigated across a range of stream water nutrient concentrations and light conditions by deploying 600 nutrient diffusing substrates (NDS) into 15 streams in July 2012 (Fig. 2). Streams were chosen to represent a gradient of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) concentrations at base flow.

Nutrient diffusing substrates were constructed of 32 ml plastic Polycon cups with a 9.5 mm diameter hole drilled into the top. Cups were filled with one of four nutrient agar treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (N+P), or agar only with no nutrient amendments (control). Each cup was fitted with either a fritted glass disk (henceforth called inorganic) or a 2.5 cm x 2.5 cm cellulose (organic) sponge substrate beneath the cap. Following Pringle and Triska (2007), the nutrient agar for our N treatment consisted of 40 g of NH_4NO_3 added to 20 g of agar completely dissolved in 1 L of deionized water; the P treatment was 87.1g of K_2HPO_4 added to 20 g of agar completely dissolved in 1 L of water; and the N + P treatment was 87.1 g of K_2HPO_4 added to 40 g of NH_4NO_3 completely dissolved in 30 g of agar and 1L of water. The control was 20 g of agar completely dissolved in 1 L of water.

Eight NDS were secured to 2.5 cm x 7.6 cm x 31 cm untreated wooden boards so that each array contained 1 complete set of nutrient treatments for both inorganic and organic substrates (1 array = 8 different treatments-substrate combinations). Five NDS arrays were deployed into each study stream along a 50 m stream reach constituting both riffles and pools. Arrays were attached to rebar secured to the streambed so that they remained submerged throughout the duration of the experiment (Fig. 2). Some study reaches required that a wire grate be secured around the NDS to reduce damage by livestock. All NDS were cleaned of debris and sediment multiple times per week.

Water samples were taken weekly during the 21-day NDS and consumer exclosure experiments (*see below*) at base flow whenever possible and never during the peak of high flow events. Water samples were filtered through 0.45μm nitrocellulose membrane filters into acid-washed polyvinyl scintillation vials, stored on ice and transported back to the lab where they were frozen until analysis for dissolved inorganic nitrogen (DIN) via a Alpkem Rapid Flow Analyzer 300 and soluble reactive phosphorus (SRP) using spectrophotometric methods by the UGA Analytical Chemistry Laboratory. Canopy cover was measured above the NDS arrays using a densiometer during the second and third weeks of the experiment.

Metabolism

After 19-21 days of incubation, NDS were collected from the field, stored in coolers, and transported to a walk-in cooler for processing within 48 hours (Fig. 2). Substrates were placed into 50 ml plastic centrifuge tubes filled with filtered stream water of a known dissolved oxygen (DO) concentration. Tubes were visually examined and any observed air bubbles were removed. Three controls of filtered stream water were included for each stream. Dissolved oxygen (DO) concentration was measured in each tube with a YSI 5100 meter attached to a 5010 BOD probe at 14 ºC after incubating the substrates under Aqueon full spectrum lights for 2 hours on a shaker table at 100 rpm.

Net ecosystem production (NEP) estimates were calculated as the change in DO divided by duration of the light treatment. Dislodged sediments were decanted through pre-weighed and ashed 0.45µm glass-fiber filters, and processed along with their corresponding substrates. To measure community respiration (CR), substrates were replaced into centrifudge tubes, refilled with filtered stream water of a known DO concentration, sealed bubble free, and placed in a dark treatment on the shaker table for 2 hours after which DO was measured. Samples were then filtered as described above and substrates were wrapped in labeled foil envelopes and placed in a -80 ºC freezer until further processing.

Algal Biomass

Chlorophyll *a* extraction was modified from Steinman et al. (2007). Before being ashed, inorganic substrates were placed into 50 ml centrifuge tubes, filled with 10 ml of 90% buffered acetone then placed in a freezer for approximately 20 hours. Samples were then removed and centrifuged at 3500 rpms for 20 minutes. Three ml of chl *a* extract was then placed in a cuvette and optical density was measured at the 630, 647, 665 and 750 nm wave lengths. Samples with readings ≥ 0.005 at the 750 nm wavelength were diluted by removing 1 ml of extract and replacing it with 1 ml acetone until the 750 nm values were < 0.005; final values were corrected for serial dilutions. Extracts were acidified with 0.1 ml of HCL, gently agitated and allowed to sit for 60 seconds before being reread at 665 and 750 nm wavelengths to correct for phaeophyton. Chl *a* mg L^{-1} was calculated following Lorenzen's Pheaopigment-corrected Chl *a* and Phaeo *a* method (EPA 1997) and then converted to areal estimates of algal biomass (mg Chl *a* m^2). Substrates were dried at 60 ºC then stored in the freezer until processing for AFDM.

Ash Free Dry Mass

Substrates were removed from the freezer, dried at 60 ºC for at least 24 hours, weighed, ashed in a muffle furnace for 4 hours at 500 ºC and then reweighed (inorganic substrates were processed for chl *a* before being dried and ashed (*see below*). Ash free dry mass (AFDM) was calculated as AFDM = dry wt. - ashed wt., and NEP and CR were calculated as the change in O_2 per hour divided by AFDM, and expressed as mg O_2 g $AFDM^{-1}$ hr^{-1}. Gross Primary Production (GPP) was calculated as the sum of NEP and CR.

Calculations and Statistical Analyses

The Response Ratio (RR) for each nutrient treatment on each array was calculated by dividing response values for each treatment (N, P, N+P) by the value for the control of that array. The natural log of the Response Ratio (LnRR) was calculated and values greater than zero were interpreted as evidence of nutrient limitation.

One-tailed, one-sample Student T-Tests were conducted on the LnRR of each substrate and nutrient treatment to assess nutrient limitation of algal biomass, GPP and CR. Outliers identified as greater than 2.5 standard deviations of the mean and were removed. Linear mixed-effects models were run using the LME4 package in R (R Core

Team 2013) to assess the strength of water chemistry and light availability (measured as canopy cover above the array) effects on responses. Water chemistry and Arcsin transformed canopy cover were scaled and centered using the “scale” function in R, and used as predictive parameters in the model. Stream identity was treated as a random effect in the model to account for the lack of independence of model residuals (Bates et al. 2014). Confidence intervals of model parameters were estimated with the Wald method. Marginal and conditional R^2 values of models were obtained using methods outlined in Nakagawa and Schielzeth (2013), and reported R^2 values are marginal R^2 values unless otherwise noted. To assess how nutrient addition influenced the relationship of CR to GPP on both inorganic and organic substrates, Analysis of Covariance (ANCOVA) was conducted on measured (not LnRR) values. Residuals were examined to identify outliers and outliers were removed if they exerted high leverage on the model and removal improved the normality of the residuals.

Experiment II: Consumer Exclosures

In July 2013, we selected nine streams to assess the microbial response to water column nutrient concentrations on organic substrates in the absence of consumers. Consumer exclosures were created using PVC rain gutter enclosed in 250µm mesh and 2.5 cm x 2.5 cm sponges were attached to glass microscope slides within the enclosures. At seven and 17 days, we removed substrates from the exclosures, placed them in centrifuge tubes filled with stream water and transported them to the lab in coolers. Substrates were then placed in BOD bottles in the dark and DO was measured every five minutes for approximately 45 minutes to obtain a rate of oxygen consumption. Substrates were then ashed as described above, and respiration rate was calculated as mg O_2 g AFDM^{-1} hr^{-1}. We regressed respiration rate against mean water chemistry using linear regression of sample means.

Results

Severe flooding occurred during the course of our NDS study resulting in the loss of many NDS during the first week and in subsequent weeks. We replaced any NDS lost or damaged within the first week. We did not replace NDS was lost after the 1st week. In addition, consumers such as crayfish, macroinvertebrates, and snails were frequently observed on our NDS arrays throughout the duration of the experiment, and potentially affected observed responses. After analyzing our water chemistry data we found strong correlations between molar N:P and DIN, and therefore did not use N:P as a predictor variable. Watershed area ranged between 0.5 and 41 km^2 and base flow discharged ranged from 2.3 to 374.6 L s^{-1} (appendix A).

To interpret differences in responses measured on our two substrate types, we assumed organic substrates contained predominately heterotrophic communities, while inorganic substrates were colonized predominately by autotrophic communities. This distinction allowed us to consider our results in terms of carbon production and sequestration by autotrophic communities compared to heterotrophic communities.

Production, respiration and limitations of algal biomass across the landscape

High within-stream variation was observed on both organic and inorganic substrates for GPP and CR metrics, which we attribute largely to high spatial variability of sedimentation, scour, and herbivory within individual streams. Analyzed as the mean lnRR from each stream, only 17% of the streams showed evidence of N limitation of GPP on inorganic substrates, while P and NP limitation was observed in 58% and 54% of the streams respectively (Table 2), indicating more frequent P limitation of GPP than N limitation. Community respiration was co-limited or P limited on inorganic substrates in 46% and 54% of streams, respectively. On organic substrates, one stream showed N limitation of GPP, while CR was limited by P or NP in 18% of the streams (Table 2). Collectively, this supports a conclusion of reduced frequency of nutrient limitation on organic substrates compared to inorganic substrates but some evidence for P limitation of heterotrophic communities. Algal biomass showed N limitation in 40% of the study streams, while 33% showed P limitation, and 67% indicated co-limitation supporting a conclusion of widespread co-nutrient limitation of algal biomass (Table 2).

Nutrient additions increased GPP on inorganic substrates over controls. GPP averaged 2.5 mg O_2 g AFDM^{-1} hr^{-1} on control inorganic substrates, and P and N+P treatments increased GPP over controls by 0.3 and 0.4 mg O_2 g AFDM^{-1} hr^{-1}, respectively (Table 3). Mean GPP was lower on organic substrates (1.2 mg O_2 g AFDM^{-1} hr^{-1} on controls; Table 3) compared with inorganic substrates. Gross primary production on organic substrates was similar between the control and P treatment, but GPP was 42% lower with the N treatment (Table 3). Using each NDS lnRR as an independent data point, one-sample, one-way t-tests indicate that GPP on inorganic substrates was significantly limited by P availability (LnRR Mean = 0.14, $t(44) = 2.17$, $p = 0.02$) but significant nutrient limitation of GPP was not observed on organic substrates (Figure 3). Community respiration on inorganic substrates averaged 1.0 mg O_2 g AFDM^{-1} hr^{-1}, 67% higher than on organic substrates (0.6, Table 3). T-test results indicated no significant landscape level response in CR rate due to nutrient enrichment on inorganic or organic substrates (Figure 3).

Algal biomass responded positively to increased nutrient availability. Algal biomass averaged 41.0 mg m^{-2} of chl *a* on inorganic-control substrates, with similar values for the P treatment (39.1 mg m^{-2}). Substantially higher biomass was observed on the N treatment (50.1 mg m^{-2}), while algal biomass receiving the N+P addition (64.1 mg m^{-2}) increased almost 60% over controls. T-tests indicated that algal biomass was significantly co-limited by N+P (lnRR = 0.27, t(60) = 3.48, $p < 0.001$; Figure 3), but not significantly limited by N or P alone. While not statically significant at $\alpha = 0.05$, the algal biomass response to N was statistically significant at $\alpha = 0.1$., and may be biologically significant.

How do light and ambient nutrients influence GPP, CR and algal biomass?

GPP

Canopy cover alone was the top model for predicting GPP on inorganic substrates receiving both the P and NP treatments. We found that canopy cover had a negative effect on GPP when P limitation was alleviated, although confidence intervals for the canopy cover effect marginally crossed zero (-0.4472, 0.0578) and canopy cover alone only accounted for 5% ($R^2 = 0.05$) of the variation in GPP. Canopy had no discernable affect on GPP in the N+P treatment ($R^2 = 0.02$; Table 4). On inorganic substrates receiving N, our models were moderately predictive ($R^2=0.34$), indicating increasing water column DIN and SRP reduced the observed difference between the treatments and controls, and that canopy exerted a positive influence on GPP. Our models were only moderately to weakly predictive of GPP on organic substrates, with R^2 values between <0.01 and 0.22.

CR

Canopy cover substantially positively influenced community respiration rate on inorganic substrates receiving N additions, and increasing water column SRP reduced the difference between the treatment and control, indicating increased respiration rate as shading increased. Canopy cover was negatively correlated with CR on inorganic substrates receiving the P treatment ($R^2 = 0.10$), and the effect of increasing water column DIN was positive, indicating nitrogen stimulation with increased P availability.

Canopy cover was included in all best-supported models for responses of CR on organic substrates, but confidence intervals for the effect of canopy crossed zero. The response to water column nutrients was most notable on organic substrates, for which including water column nutrients improved model fit (R^2) in all cases. In the P and N+P treatment, increasing water column DIN decreased the differences in CR between the treatment and control. Models for CR on organic substrates were only weakly predictive ($R^2 = 0.05 – 0.21$); we interpreted these results as indications of *in situ* alleviation of nutrient limitation but also as suggesting that factors other than or in conjunction with nutrients can exert large influences on CR.

Algal Biomass (Chl *a*)

Canopy cover was included in all best-supported models for algal biomass models and our strongest model included the P treatment and a generally positive influence of water column DIN and SRP ($R^2 = 0.17$) compared with controls. Overall we found that canopy cover had a neutral effect on algal biomass across single- and duel-nutrient additions although model strength was relatively weak ($R^2 = <0.01 – 0.17$; Table 4).

Does nutrient addition change carbon balance?

Regression lines for GPP and CR from all treatments were above the 1:1 ratio indicating that activity on these substrates under test conditions was net autotrophic, on both organic and inorganic substrates (Figure 4). The relationships between CR and GPP were significantly correlated in all nutrient treatments ($P < 0.01$) on both inorganic and organic substrates. On inorganic substrates N addition significantly reduced GPP relative to CR compared with control ($P = 0.02$) while the NP treatment significantly increased GPP ($P < 0.01$) as indicated by difference in intercept. No differences in slope were found between the nutrient treatments and controls on inorganic or organic substrates.

In the absence of consumer pressure, do nutrients influence microbial activity?

Results from the consumer exclosures indicated a significantly positive correlation between water column DIN and community respiration at both the 7 and 17-day incubations (Figure 5; $P = 0.006$, adj. $R^2 = 0.64$; $P = 0.002$, adj. $R^2 = 0.73$, respectively). The trend however, was strongly influenced by a single high DIN stream (375 µg L^{-1}) at both time periods. Removal of this data point resulted in a significant positive trend at 7 days ($P = 0.05$), but no significant trend at 17 days (not shown). However, we found no significant correlation between respiration rate and stream water SRP, although removing the highest DIN stream from the analysis resulted in significant correlations between respiration rate and SRP in the 17-day ($P = 0.03$, adj. $R^2 = 0.48$) incubation but not in the 7-day incubation.

Discussion

Nutrient limitation is dependent on carbon availability and light

We found significant landscape scale P limitation of GPP but co-limitation (N+P) of algal biomass on inorganic substrates in the NDS study, in addition to a positive correlation between CR on organic substrates and water column gradients of both DIN and SRP in our consumer exclosure study. These results indicate that carbon availability and substrate characteristics (e.g., erodability, palatability) can exert a strong influence on benthic biofilm nutrient demands and detectability of responses to additional nutrients.

Our model results indicate that on inorganic substrates under conditions of increased nitrogen availability, canopy cover exerts a positive influence on respiration rate which we interpretive to indicate increased heterotrophic activity. However, in contrast to other studies, we did not find a positive landscape scale biofilm metabolism response to nutrient enrichment on organic substrates (Hoellein et al. 2010; Johnson et al. 2009), which we interpret as nutrient saturation for heterotrophic pathways at the time interval tested, approximately 21 days. Canopy cover was included in all models for GPP, CR and algal biomass, but the effect varied widely between substrate types and nutrient treatments.

Algal biomass, GPP and CR responses to enrichment

Algal biomass is the most widely used metric to assess nutrient enrichment, but algal biomass is also influenced by light, water chemistry and temperature. Additionally, algal types (bacillariophyta, chlorophyta, cyanobacteria, etc.) respond differently to these influences. Algal biomass in our study streams varied widely across nutrient treatments, from less than 5 to over 500 mg m^{-2}, and while algal biomass was higher than levels reported in some studies (Hagen et al. 2010; Hoellein et al. 2010), our values are within ranges reported by in Dodds et al. (2002) in their survey of literature values. Our findings that algal biomass is co-limited by N+P are supported by other studies and contribute to the growing body of evidence that dual nutrient reduction strategies are necessary to manage algal biomass accural in freshwater systems (Dodds et al. 2002; Elser et al. 2007; Francoeur 2001; Harpole et al. 2011; Tank and Dodds 2003). In our models, increasing water column nitrogen (DIN) positively influenced algal biomass accrual in both the N and P treatments, compared with control treatments. Surprisingly, while canopy cover was included in all the top modes for algal biomass, canopy cover was not strongly predictive of algal biomass, which may reflect limitations on our ability to measure benthic light availability and differentiate algal communities between our study streams.

Similar to algal biomass, phosphorus limitation of GPP has also been reported by Johnson et al. (2009) on inorganic substrates, but the effects of nutrients can be overshadowed by the effect of canopy (Mosisch et al. 2001), sedimentation and scour. Our results were similar to those of Johnson et al. (2009), where between 50% (agricultural) to 75% (reference and urbanized) of their study streams showed no evidence of nutrient-limited GPP on organic substrates. Furthermore, the effect of sedimentation, which was very high in some of our study streams, likely reduced the magnitude of any autotrophic response. Similarly, the effect of grazers on our substrates may have reduced our ability to detect differences between nutrient treatments and controls if consumers preferentially consumed nutrient enriched biofilms.

We found little evidence that CR was strongly nutrient limited on inorganic or organic substrates, however our regression analysis showed a positive influence of canopy cover on CR in the N treatment on inorganic substrates, possibly indicating increased heterotrophic activity within the autotrophic biofilm matrix. On inorganic substrates alleviated of P limitation, canopy cover exerted a negative effect on CR. These results may be indicative of changes in biofilm community composition between the nutrient treatments, or canopy cover may be acting as a surrogate for unmeasured stream variables such as scour and turbidity that influenced community composition and response to nutrients.

Consumer Exclosures

At in situ concentrations, both increasing water column SRP and DIN increased heterotrophic activity. While these data support our hypothesis that microbial respiration rate is dependent on water column nutrients, the disparity between the exclosure and NDS study supports a conclusion that consumers and scour can strongly influence carbon retention and export in stream ecosystems, and can obscure the observable influences of surface water nutrients on organic substrates. Because we did not set out to quantify scour or herbivory, the magnitude of the effects are not known.

Do the effects of nutrient addition on carbon balance depend on community type?

On our inorganic substrates, we found that N addition increased the relative amount of CR compared with GPP (lower intercept) over control conditions, while N+P addition increased the relative amount of GPP compared with CR (higher intercept) indicating that nutrients can change carbon balance. Surprisingly, we did not find significant differences in the slope of the GPP:CR relationship under different nutrient treatments, and we report a relatively static GPP to CR relationship throughout the range of GPP and CR measured in our test conditions. Finally, while we did not find greater CR rate on organic substrates at the landscape level, several studies have shown that nutrient enrichment can increase respiration rates of heterotrophic microbes (Manning et al. 2016, Gullis et al 2004) and induce changes in autotrophic and heterotrophic biofilm community composition (Moller et al. 1997; Sekar et al. 2002), which may provide a mechanistic explanation for the observed variability in response to nutrients.

Management implications

With the expanding understanding of the interactions between light and nutrients on the trophic state of streams, and with the increased realization of the importance of autotrophic and heterotrophic communities in affecting stream carbon dynamics (Dodds 2007; Dodds and Cole 2007; Rosemond et al. 2015), managers could develop nutrient criteria (N & P) to aid in the management of algal biomass and carbon resources. Our results indicate that when canopy cover is above approximately 80%, algal biomass can be substantially inhibited (Fig 6) and streams with lower canopy cover appear to be more sensitive to enrichment by N and P. The differences we find in nutrient limitation between the structural (algal biomass) and functional metrics (GPP / CR) reflect the complicated relationships between production and community composition in the biofilms.

Because of the interacting effects of scour, grazing, and canopy cover, algal biomass alone cannot capture the full effects of nutrient enrichment, nor predict the accelerated loss of carbon from streams due to enrichment. Thus, developing nutrient criteria based on functional rates may facilitate the assessment of stream impairment and the retention of basal resources in lotic systems.

Organic Matter Dynamics

Our results indicate that canopy cover can have a substantial suppressive effect on GPP and CR in cases of limitation by N or P, however, when limitation by both nutrients is alleviated (N+P treatment) the suppressive effect of canopy is reduced. While the determination of what constitutes an excess of nutrient can vary by ecoregion (Tank and Dodds 2003), the differences between autotrophic and heterotrophic responses to enrichment observed in this study indicate that alleviation of nutrient limitation for heterotrophic pathways occurs at a lower threshold than for autotrophic pathways. Additionally, nutrient enrichment can influence prey body size (Davis et al. 2010b) and predator-prey dynamics (Davis et al. 2010a) by altering the quality and quantity of these resources. Therefore reductions in carbon stocks can negatively impact freshwater ecosystem health by reducing the availability of energy to higher trophic level and enrichment may have economic implications related to the diminishment of ecosystem services.

Flooding and high water events can influence algal biomass (Biggs 1995) and stocks of organic matter. The flooding that occurred during this experiment had an unknown impact on our results, and we attribute some of the variability we observed in the data, especially on organic substrates, to the effects of flooding. A larger flood effect on organic substrates, combined the effects of herbivory and detritivory, may have reduced our abilities to measure treatment effects. Additionally, crayfish and other shredders were frequently observed on organic substrates and likely reduced our ability to observe differences between treatments and controls. With the increasing need to conduct studies in urbanized and semi-urbanized watersheds and the frequency of extreme weather patterns caused by climate change; additional investigation into the effects of flooding and scour on biofilm community composition and biomass is warranted. Furthermore, methods to quantify the effect of consumers would elucidate interactions between nutrient enriched biofilms and consumers effects on organic substrates.

Summary

With fresh water becoming increasingly enriched with nutrients, future studies of nutrient limitation on organic substrates may benefit from including repeated measurements of functional metrics over time to better assess successional changes in responses to enrichment. The effects of nutrients and light on stream metabolism and carbon storage vary widely between ecoregions, owing to variations in climate, basin characteristics, and biotic community (Bernot et al. 2010). But because urbanization acts as a homogenizing force on aquatic systems via increasing nutrient concentration and alleviating light limitation, efforts to increase stream shading may be effective in helping to reduced the effects of nutrient loading. Our results highlight the differential responses of functional and structural ecosystem metrics to nutrient enrichment, and elucidate the important regulatory effect of canopy cover on GPP and CR. The maintenance of canopy cover at greater than 80% may prevent nuisance levels of algal biomass accrual in streams under moderately enriched conditions. However, because of the interactions between nutrients and light, reductions in nutrients alone may not be sufficient to maintain algal biomass below nuisance levels in open canopy systems, and our results must be taken in the context of the extreme weather experienced during the study period.

With the convergent pressures of climate change, urbanization, population growth and the associated increased demand for freshwater, there is a clear urgency for the tools necessary to mange freshwater resources to preserve ecosystem service and ecological integrity.

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Table 2.1. Measured means ± (standard error) for nutrient diffusing substrates deployed into 15 2^{nd}-3^{rd} order streams for algal biomass, primary production (GPP) and community respiration (CR) on organic and inorganic substrates for each nutrient treatment (N= nitrogen, P = phosphorus, NP = nitrogen + phosphorus) plus control (C). Each stream had five replicates deployed but between 0-5 of the replicates were able to be retrieved. NA indicates missing data.

Stream name	Algal Biomass (Chl *a*, mg m^{-2})				Inorganic GPP (mg O_2 g AFDM-^{1} hr^{-1})				Inorganic CR (mg O_2 g AFDM-^{1} hr^{-1})				Organic GPP (mg O_2 g AFDM-^{1} hr^{-1})				Organic CR (mg O_2 g AFDM-^{1} hr^{-1})			
	C	N	P	NP	C	N	P	NP	C	N	P	NP	C	N	P	NP	C	N	P	NP
Blacks Branch	6.7 (0.7)	9.1 (4.4)	10.6 (4.2)	10.5 (1.9)	0.71 (0.32)	NA	NA	0.91 (NA)	0.7 (0.03)	NA	0.18 (NA)	2.05 (1.99)	1.23 (0.10)	0.36 (0.13)	1.20 (0.16)	0.38 (0.16)	0.60 (0.04)	0.19 (0.10)	0.43 (0.11)	0.09 (0.04)
Caler	101.1 (31.7)	82.5 (28.4)	115.3 (44.9)	156.7 (65.7)	4.15 (0.40)	1.83 (0.50)	5.23 (0.94)	2.74 (0.52)	1.75 (0.15)	0.65 (0.11)	2.67 (0.75)	1.17 (0.24)	1.22 (0.33)	0.50 (0.11)	0.96 (0.15)	0.92 (0.28)	0.70 (0.19)	0.30 (0.07)	0.60 (0.10)	0.54 (0.14)
Cowee	24.1 (3.9)	16 (1.8)	20.3 (4.9)	15.8 (5.9)	3.43 (0.77)	1.66 (0.54)	2.22 (0.19)	3.78 (0.79)	1.14 (0.28)	0.70 (0.30)	0.57 (0.17)	0.93 (0.28)	0.65 0.18)	0.78 (0.27)	0.66 (0.01)	0.80 (0.16)	0.29 (0.17)	0.34 (0.08)	0.37 (<0.01)	0.42 (0.11)
Crawford	7.5 (1.2)	2.9 (1.0)	4.5 (1.4)	3.0 (1.8)	3.21 (0.49)	2.08 (0.54)	3.09 (0.41)	2.03 (0.12)	1.65 (0.36)	1.48 (0.27)	1.68 (0.17)	1.06 (0.06)	NA	NA	0.70	0.48 (0.06)	NA	NA	0.29 (NA)	0.26 (0.03)
Dalton	30.5 (8.3)	22.6 (8.3)	18.8 (9.6)	43.3 (26.5)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Darnell	62.2 (17.3)	104.7 (29.2)	60.3 (18.2)	142.4 (38.7)	2.11 (0.86)	2.15 (0.35)	2.20 (0.44)	3.65 (0.39)	0.71 (0.23)	0.55 (0.14)	0.60 (0.12)	0.83 (0.09)	0.82 (NA)	NA	NA	1.10 (NA)	0.21 (0.02)	0.35 (NA)	NA	0.35 (NA)
Frog	15.1 (4.9)	119.6 (97.2)	53.1 (33.7)	113.0 (96.1)	1.97 (0.29)	0.90 (0.01)	2.02 (0.22)	1.75 (0.39)	0.49 (0.18)	0.45 (0.09)	0.65 (0.18)	0.46 (0.10)	1.68 (0.25)	0.70 (0.12)	1.90 (0.27)	1.06 (0.36)	0.73 (0.15)	0.28 (0.06)	0.68 (0.17)	0.32 (0.10)
Howard Branch	3.8 (1.2)	4.5 (0.8)	5.3 (0.9)	3.0 (0.8)	2.17 (0.30)	1.22 (0.12)	2.73 (0.15)	3.97 (0.37)	0.76 (0.22)	0.17 (0.04)	0.65 (0.21)	1.51 (0.46)	0.69 (0.15)	0.40 (0.10)	0.68 (0.12)	0.48 (0.11)	0.31 (0.09)	0.16 (0.06)	0.22 (0.10)	0.14 (0.04)
Jerry	5.0 (3.3)	3.8 (<0.1)	8.3 (3.0)	9.4 (1.9)	1.32 (0.32)	0.72 (0.43)	1.39 (0.55)	1.08 (0.37)	0.64 (0.18)	0.31 (0.18)	0.64 (0.19)	0.58 (0.05)	1.40 (0.38)	0.74 (0.36)	1.56 (0.63)	0.90 (0.44)	0.75 (0.22)	0.38 (0.17)	0.82 (0.39)	0.46 (0.27)
Jones	25.2 (7.7)	35.4 (15.7)	20.3 (3.9)	24.1 (6.1)	1.96 (0.41)	1.43 (0.58)	2.35 (0.43)	2.03 (0.37)	1.46 (0.31)	1.28 (0.38)	1.68 (0.34)	1.26 (0.14)	1.35 (0.19)	0.68 (0.11)	1.41 (0.30)	1.36 (0.26)	0.92 (0.13)	0.42 (0.07)	0.93 (0.20)	0.83 (0.20)
Mica	50.6 (9.4)	65.5 (41.4)	41.4 (12.7)	55.0 (5.9)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Shope	117.9 (58.8)	122.0 (31.8)	104.0 (41.3)	210.2 (39.8)	2.29 (0.21)	1.95 (0.30)	2.05 (NA)	5.02 (0.68)	0.68 (0.17)	0.54 (0.14)	0.19 (NA)	1.39 (0.44)	0.99 (0.12)	0.97 (0.13)	0.82 (0.06)	0.80 (0.22)	0.39 (0.07)	0.36 (0.07)	0.34 (0.04)	0.32 (0.13)
Skeena	41.4 (19.2)	58.8 (40.2)	21.1 (7.4)	49.0 (30.4)	1.62 (0.49)	2.03 (0.42)	2.35 (0.34)	2.25 (0.23)	0.78 (0.25)	0.94 (0.25)	0.62 (0.13)	0.42 (0.07)	1.25 (0.13)	1.00 (0.19)	1.21 (0.30)	1.61 (0.10)	0.65 (0.09)	0.41 (0.09)	0.54 (0.17)	0.61 (0.02)
Tessentee	33.9 (11.2)	74.4 (14.2)	97.0 (32.3)	43.3 (21.1)	3.12 (0.64)	1.30 (0.39)	2.28 (0.20)	1.99 (0.43)	1.53 (0.34)	0.79 (0.33)	1.08 (0.13)	0.73 (0.20)	1.39 (0.26)	0.76 (0.11)	1.23 (0.17)	1.29 (0.19)	0.74 (0.09)	0.39 (0.04)	0.60 (0.08)	0.72 (0.06)
Watauga	14.3 (5.1)	23.4 (7.6)	17.3 (6.0)	41.1 (20.0)	2.95 (0.44)	3.70 (0.89)	4.88 (0.47)	3.62 (0.83)	0.96 (0.31)	1.66 (0.48)	1.83 (0.19)	1.00 (0.31)	0.96 (0.26)	0.49 (0.12)	1.12 (0.26)	0.88 (0.27)	0.53 (0.15)	0.31 (0.11)	0.67 (0.17)	0.63 (0.20)

Table 2.2. Stream response to nutrient enrichment calculated as the mean natural log of the response ratio from each array, averaged by stream. Mean ratios > 0 were interpreted to indicate a stream- level response to nutrient treatments and are indicated with a “*”, means ≤ 0 are noted with a “-”, samples size ranged up to 5 RRs; missing data are indicated with (na). Reported water chemistry is the mean from 3 weekly samples, canopy cover by stream averaged from all arrays.

	Water Chemistry (μg L^{-1})		Canopy Cover	Algal Biomass			Inorganic GPP			Inorganic CR			Organic GPP			Organic CR		
	DIN	SRP	mean %	N	P	NP	N	P	NP	N	P	NP	N	P	NP	N	P	NP
Percent of streams with a mean lnRR > 0				40	33	67	17	58	54	15	54	46	1	45	18	17	18	18
Blacks Br.	377.5	12.9	**0.0**	-	*	*	na	na	-	-	*	*	-	-	-	-	-	-
Caler	64.7	11.9	0.0	-	*	*	-	*	-	-	*	-	-	-	-	-	-	-
Cowee	63.3	6.8	67.0	-	-	-	-	-	*	-	-	-	*	*	*	*	*	*
Crawford	477.4	5.5	91.4	-	-	-	-	-	-	*	*	-	na	na	na	na	na	na
Dalton	47.8	8.9	73.7	-	-	*	na	na	na	na	na	na	na	na	na	na	na	na
Darnell	125.4	7.1	81.3	*	-	*	-	-	*	-	-	*	na	na	na	*	na	na
Frog	449.3	9.0	65.4	*	*	*	-	*	-	-	*	*	-	*	-	-	-	-
HWBR	261.5	11.3	94.4	-	-	-	-	*	*	-	-	*	-	*	-	-	-	-
Jerry	466.5	17.2	39.8	-	-	*	-	*	-	-	*	-	-	-	-	-	-	-
Jones	84.1	14.9	90.3	*	-	-	-	*	*	-	*	-	-	-	-	-	-	-
Mica	50.3	12.5	44.8	*	-	*	na	na	na	na	na	na	na	na	na	na	na	na
Shope	56.1	9.1	0.0	-	-	*	-	-	*	-	-	*	-	-	-	-	-	-
Skeena	203.4	5.8	87.6	-	-	-	*	*	*	-	-	-	-	*	*	-	-	-
Tessentee	648.3	9.6	67.0	*	*	*	-	-	-	-	-	-	-	_	-	-	-	-
Watauga	141.0	7.8	44.7	*	*	*	*	*	*	*	*	*	-	*	-	-	*	*

Table 2.3. Mean (standard error) algal biomass (chl *a*, mg mg^{-2}), gross primary production (GPP, mg O2 g AFDM^{-1} hr^{-1}), net ecosystem production (NEP, mg O2 g AFDM^{-1} hr^{-1}) and community respiration (CR, mg O2 g AFDM^{-1} hr^{-1}) averaged across streams. Values are for inorganic or organic substrates, receiving nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP) treatments or no nutrient addition, (control, C).

	Inorganic				Organic		
Treatment	Algal Biomass	GPP	NEP	CR	GPP	NEP	CR
C	41.01 (7.09)	2.49 (0.17)	1.36 (0.12)	1.02 (0.09)	1.19 (0.08)	0.58 (0.04)	0.61 (0.05)
N	50.13 (8.33)	1.79 (0.17)	0.83 (0.01)	0.80 (0.10)	0.67 (0.05)	0.34 (0.03)	0.32 (0.02)
P	39.06 (6.4)	2.78 (0.20)	2.01 (0.22)	1.15 (0.13)	1.15 (0.08)	0.59 (0.05)	0.56 (0.05)
NP	64.06 (11.6)	2.89 (0.21)	1.85 (0.15)	1.00 (0.12)	0.93 (0.08)	0.47 (0.05)	0.45 (0.05)

Table 2.4. LnRR LMER scaled modeled parameters of canopy cover, water chemistry dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) as predictors of ecosystem response (Community Respiration (CR), Gross Primary Production (GPP) and algal biomass (Chl *a* – inorganic only) on inorganic and organic substrates. Algal biomass was not measured on the organic substrates. Top three AIC models are listed with marginal and conditional R^2 values. Upper and lower bounds of 95% confidence intervals are shown for predictor variables.

Substrate	**Ecosystem Metric**	**Treatment**	**Model rank**	**Model code**	**Canopy 2.5%**	**Canopy 97.5%**	**DIN 2.5%**	**DIN 97.5%**	**SRP 2.5%**	**SRP 97.5%**	**Mar. R^2**	**Con. R^2**	**AICc**	**Delta AIC**	**AICc Wt**	**Cum Wt**
Inorganic	GPP	N	1	8	-0.0576	0.2812	-0.3904	-0.295	-0.436	-0.0907	0.34	0.34	62.49	0	0.65	0.65
			2	7	-0.1308	0.2182			-0.4714	-0.0908	0.24	0.27	64.33	1.84	0.26	0.91
		P	1	2	-0.4472	0.0578					0.05	0.82	62.21	0	0.57	0.57
		N+P	1	2	-0.3098	0.1485					0.02	0.23	90.83	0	0.45	0.45
			2	6	-0.2668	0.1663	-0.4365	0.067			0.08	0.22	91.61	0.79	0.3	0.75
	CR	N	1	2	0.5863	0.9195					0.66	0.68	78.33	0	0.45	0.45
			2	7	0.5675	0.8976			-0.3326	0.0519	0.68	0.7	78.9	0.57	0.34	0.79
		P	1	2	-0.4133	0.0501					0.07	0.25	76.59	0	0.53	0.53
			2	6	-0.4197	0.0278	-0.093	0.3621			0.10	0.21	78.07	1.48	0.25	0.78
		N+P	1	2	-0.3079	0.1213					0.02	0.11	95.88	0	0.49	0.49
			2	6	-0.2774	0.1485	-0.3802	0.0806			0.06	0.13	96.88	1	0.3	0.79
	Chl *a*	N	1	2	-0.1995	0.2813					<0.01	0.27	119	0	0.46	0.46
			2	6	-0.2042	0.2597	-0.0815	0.4341			0.05	0.27	119.75	0.74	0.32	0.78
		P	1	6	-0.2039	0.1704	0.106	0.5029			0.15	0.15	125.81	0	0.6	0.6
			2	8	-0.1742	0.2244	0.1111	0.5037	-0.0949	0.3419	0.17	0.17	127.13	1.32	0.31	0.92
		N+P	1	2	-0.2718	0.0486					0.04	0.04	96.39	0	0.59	0.59
Organic	GPP	N	1	7	-0.2365	0.2106			-0.5391	-0.0512	0.15	0.16	78.03	0	0.33	0.33
			2	8	-0.1866	0.2547	-0.4338	0.0328	-0.4785	0.0071	0.22	0.22	78.31	0.29	0.29	0.62
			3	6	-0.1322	0.3156	-0.5007	-0.0296			0.14	0.14	78.74	0.71	0.23	0.85
		P	1	2	-0.0884	0.1729					<0.01	0.01	41.64	0	0.58	0.58
		N+P	1	2	-0.2375	0.2209					<0.01	0.1	80.84	0	0.31	0.31
			2	7	-0.2371	0.1932			-0.4206	0.0341	0.08	0.14	80.9	0.07	0.3	0.61
			3	6	-0.1674	0.2507	-0.3579	0.0814			0.04	0.04	82.26	1.42	0.15	0.77
	CR	N	1	6	-0.3003	0.6933	-0.5523	-0.0483			0.15	0.15	86.22	0	0.32	0.32
			2	8	-0.4311	0.5683	-0.4882	0.0195	-0.4942	0.0366	0.21	0.21	86.44	0.23	0.29	0.61
			3	7	-0.5483	0.4689			-0.5675	-0.0377	0.14	0.14	86.6	0.38	0.27	0.88
		P	1	2	-0.2389	0.1508					<0.01	<0.01	64.72	0	0.49	0.49
			2	6	-0.2238	0.1588	-0.3288	-0.0775			0.05	0.05	66.07	1.34	0.25	0.75

N+P	1	6	-0.1401	0.4099	-0.5523	0.0149			0.10	0.1	97.72	0	0.33	0.33
	2	2	-0.2381	0.3726					<0.01	0.1	98.11	0.38	0.27	0.59
	3	7	-0.2599	0.3323			-0.5587	0.0632	0.09	0.14	98.49	0.76	0.22	0.82

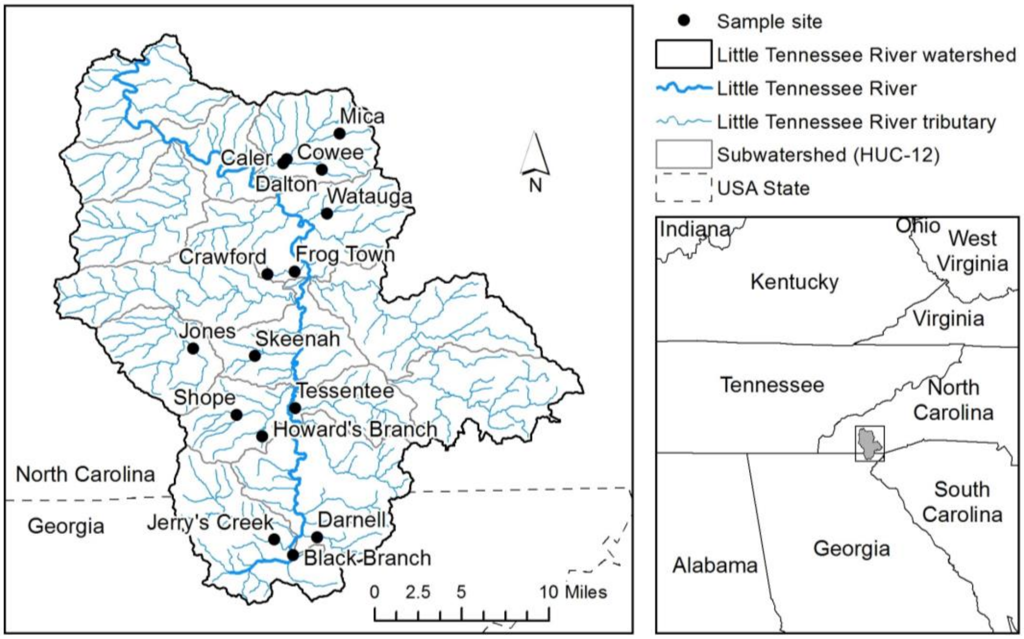


Figure 2.1. Map of the Little Tennessee River watershed, which flows north from Georgia into North Carolina. Sampling locations (black dotes) were in 2-3rd order tributaries to the Little Tennessee. Franklin, NC is the largest city in the watershed and is located at the sampling location at Frog Town Creek.

Figure 2.2. (Left) An example of a study stream showing Nutrient Diffusing Substrates array with one replicate of each nutrient treatment on both inorganic and organic substrates. Five arrays were deployed in each stream throughout a 50-meter reach. (Top Right) NDS array being removed from the stream. (Bottom Right) NDS being after deployment, note the range of conditions seen on each substrate. Photographs by J. Wood.

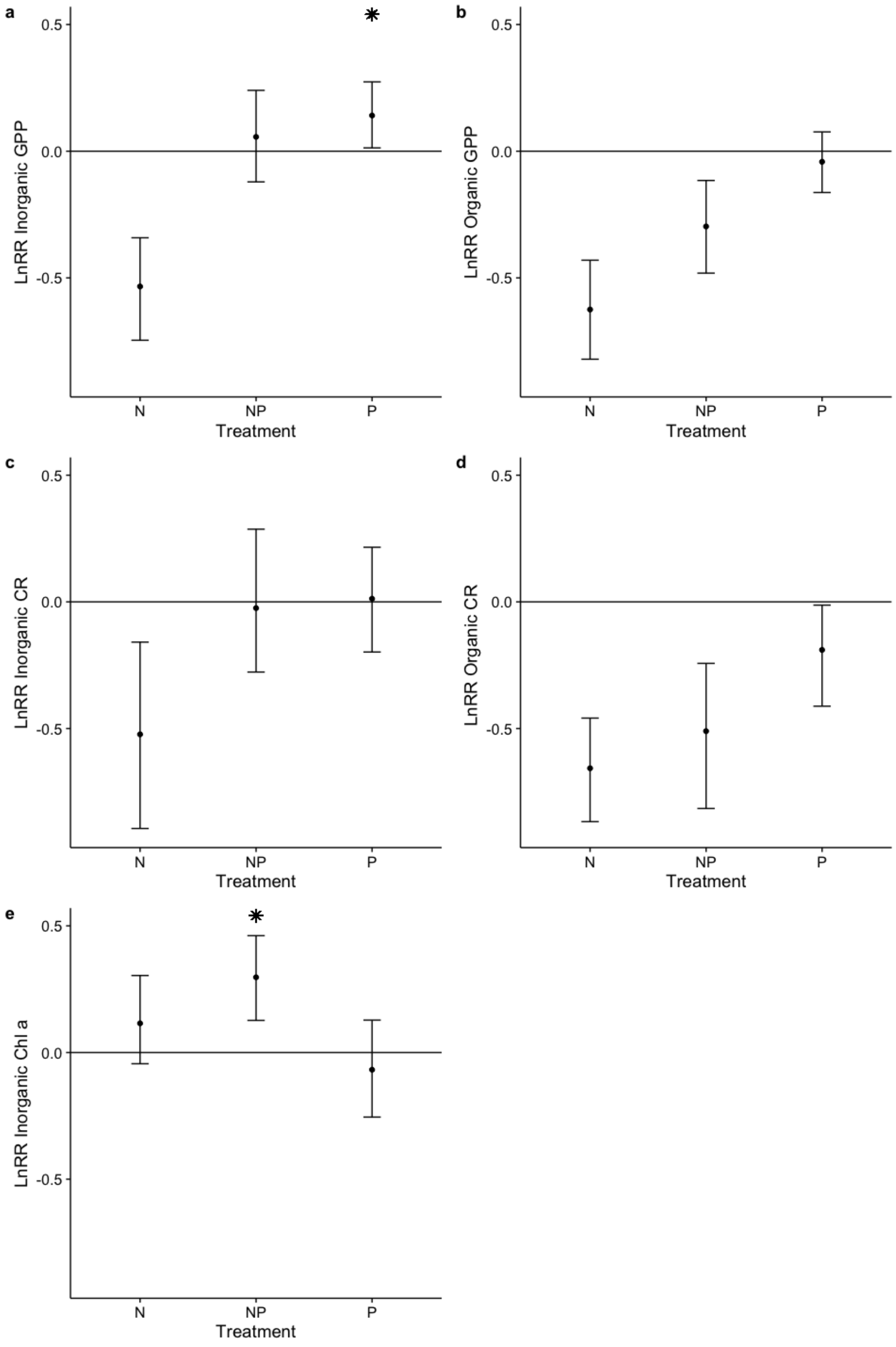
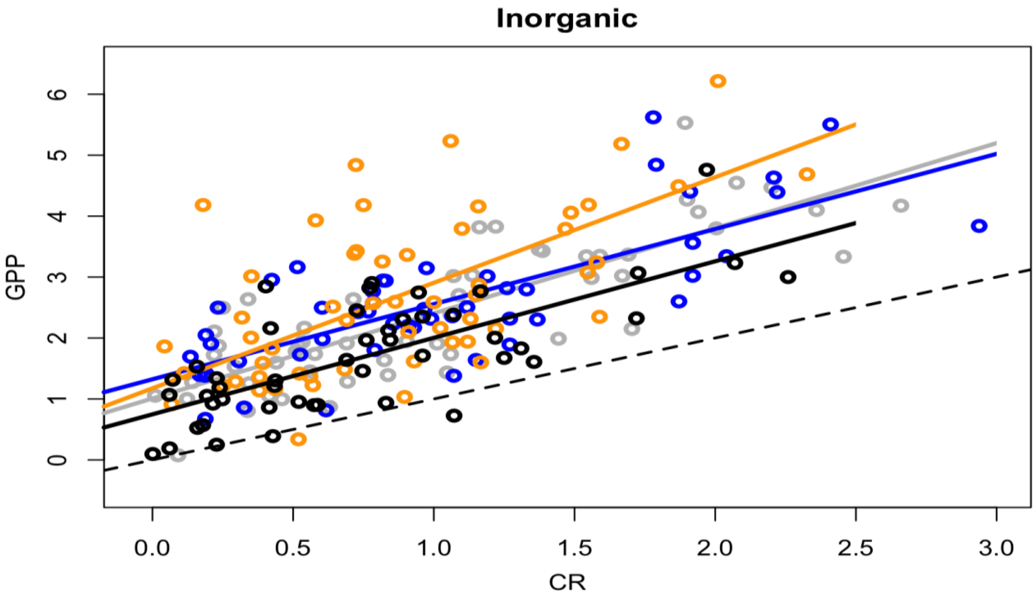


Figure 2.3. Plots show means with 95% confidence intervals of natural logged response ratio (Ln RR = ln(treatment / control)) of ecosystem responses :(a–b) gross primary production; (c–d) community respiration; and (e) algal biomass (Chl *a*). Treatments are nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP), on organic (b and d) and inorganic (a,c,and e) substrates. Asterisks indicate one-sample one-way T-tests for differences from 0 were significant at α = 0.05.



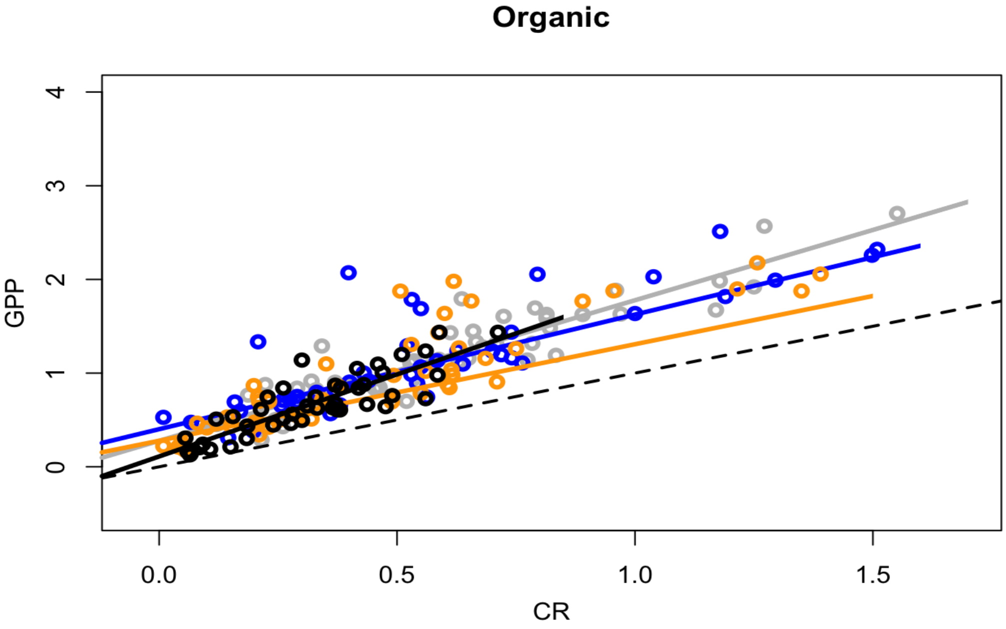


Figure 2.4. The relationship between community respiration (CR) and gross primary production (GPP) for the nitrogen (N, black), phosphorus (P, blue), N+P (NP, orange), and control (gray) treatments. CR and GPP were significantly correlated for all treatments ($P<0.001$) on both substrates. (Top) On inorganic substrates, the N intercept was significantly lower compared with control (ANCOVA, $P=0.001$) and the NP treatment (orange) had a significantly elevated intercept compared with control ($P=0.005$); no differences in slopes were detected between treatments and control. (Bottom) No differences in slope or intercept were detected between nutrient treatments and control on organic substrates. Dashed line represents a 1:1 line between CR and GPP.

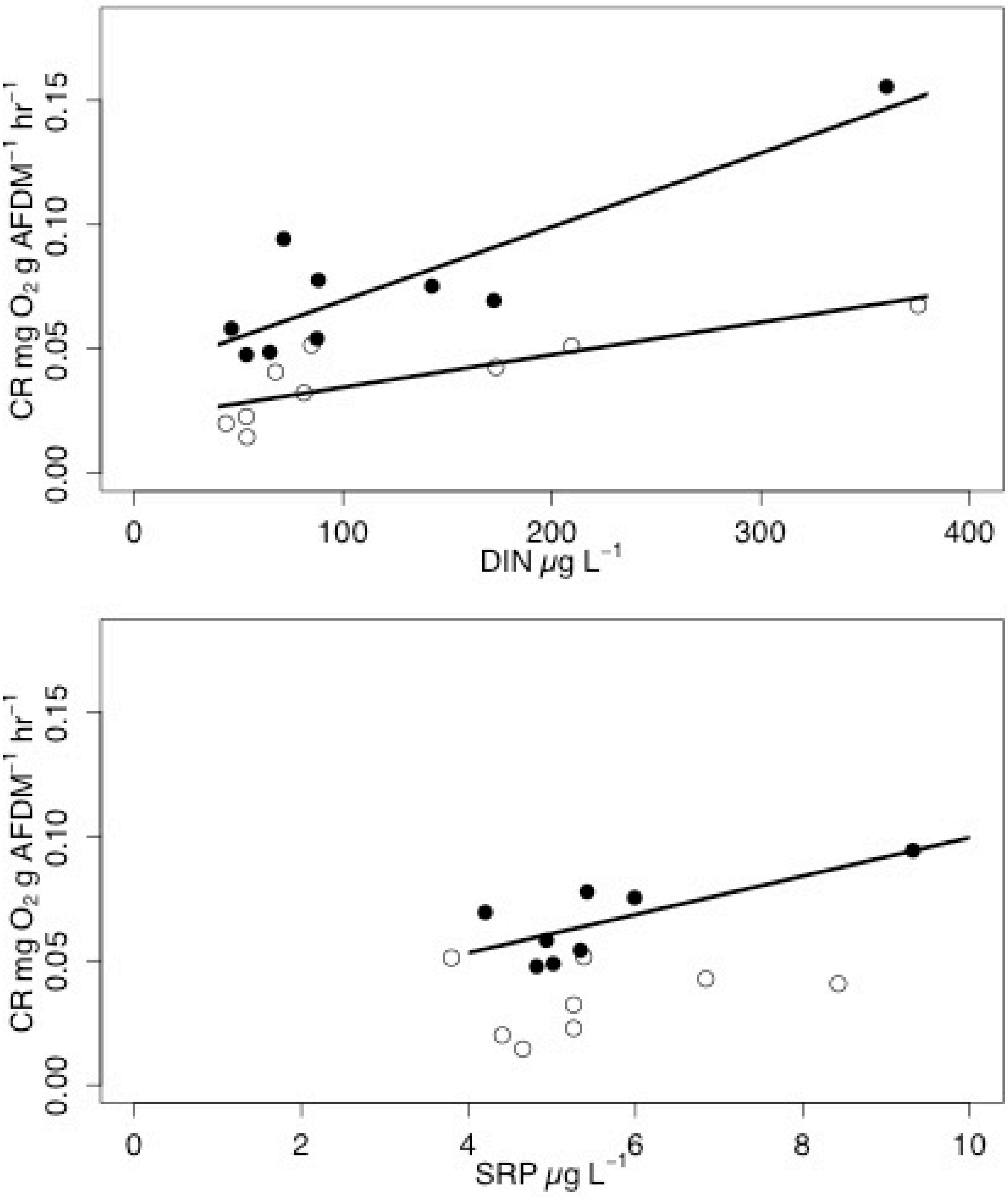


Figure 2.5. (Top) Community Respiration (CR) rate increased on organic substrates with increasing stream water DIN in both the 7-day (open circles, adj. $R^2 = 0.64$) and 17-day (closed circles, adj. $R^2=0.73$) incubations. (Bottom) Respiration rate was significantly correlated with SRP at 17-day when the highest DIN site was removed but not at the 7-day incubation.

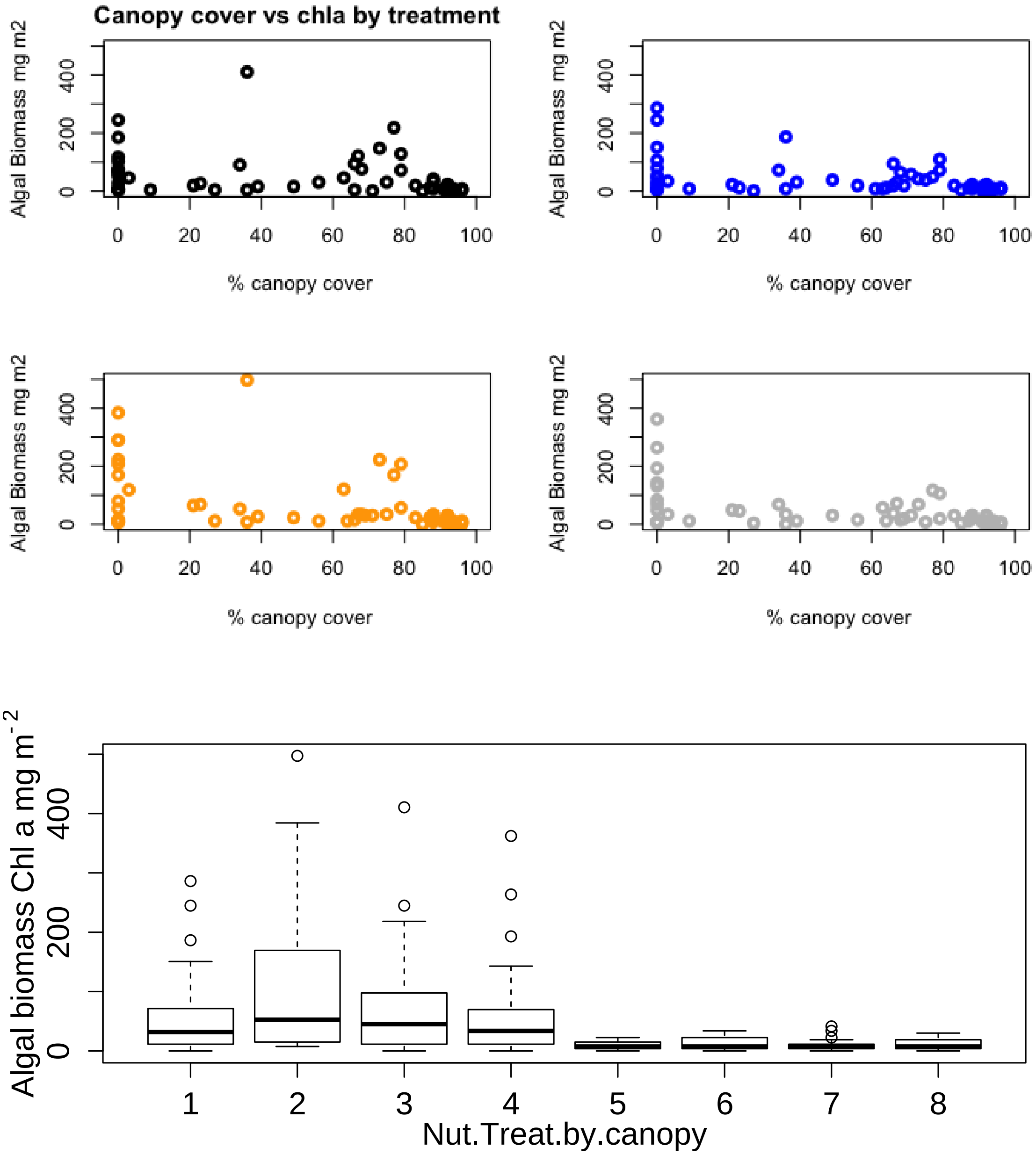


Figure 2.6. (top) Algal biomass relationship to canopy cover for nutrient treatments: nitrogen (black), phosphorus (blue), nitrogen + phosphorus (orange) and control (gray). (bottom) High levels of canopy cover (over 80%) may suppress the stimulatory effect of nutrient enrichment on algal biomass. Numbers 1-4 are samples with a canopy between 0 and 79.5%, while 5-8 represent samples with a canopy great than 79.5%. Phosphorus treatment = 1 & 5, N+P treatment = 2 & 6, N treatment = 3 & 7, control = 4 & 8.

CHAPTER 3

ECOLOGY OF THE MACROPHYTE *PODOSTEMUM CERATOPHYLLUM* MICHX. (HORNLEAF RIVERWEED), A WIDESPREAD FOUNDATION SPECIES OF EASTERN NORTH AMERICAN RIVERS[1]

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[1] Wood, J. W. and Freeman. M. C. 2017. *Aquatic Botany* 139:65-74. Reprinted here with permission of the publisher.

Abstract

Podostemum ceratophyllum, commonly called Hornleaf Riverweed, occurs in mid-order montane and piedmont rivers of eastern North America, where the plant grows submerged and attached to rocks and stable substrates in swift, aerated water. Multiple studies, mostly conducted in the southern portions of the plant's range, have shown that *Podostemum* can variously influence benthic communities in flowing waters. However, a synthetic review of the biology and ecology of the plant is needed to inform conservation, particularly because *P. ceratophyllum* is reported to be in decline in much of its range, for mostly unknown reasons. We have thus summarized the literature showing that *Podostemum* provides substantial habitat for invertebrates and fish, may be consumed by invertebrates, turtles, and other vertebrates, removes and sequesters dissolved elements (i.e., nitrogen, phosphorus, calcium, zinc, etc.) from the water column, and contributes organic matter to the detrital pool. *Podostemum* may be tolerant to some forms of pollution but appears vulnerable to sedimentation, epiphytic overgrowth, and hydrologic changes that result in desiccation, and possibly increased herbivory pressure. Much remains unknown about *Podostemum*, including aspects of morphological variation, seed dispersal, and tolerance to changes in temperature and water chemistry. Nonetheless, *Podostemum* may be considered a foundation species, whose loss from eastern North American rivers is likely to affect higher trophic levels and ecosystem processes.

Introduction

Macrophyte ecology is an active area of aquatic research and research has shown that plants influence aquatic community structure and species composition (Argentina et al., 2010b; Camp et al., 2014), nutrient cycling (Keitel et al., 2016), benthic foodwebs (Lodge, 1991) and ecosystem level processing, and the retention of elements within the system (Vila-Costa et al., 2016). However, there still exist large deficiencies in our understanding of how riverine macrophytes are influenced by land use and subsequent changes in water quality (Argentina et al., 2010a; Manolaki and Papastergiadou, 2013; Bakker et al., 2016). To maintain the ecological integrity of river systems, it is important to be able to identify stressors to riverine macrophytes and predict species persistence for a given environmental change. Here we review the available literature concerning what we believe to be the most ecologically influential macrophytes in mid-order montane and piedmont rivers of eastern North America. *Podostemum ceratophyllum* Michx., commonly called Hornleaf Riverweed, is a flowering plant (angiosperm) that grows submerged and attached to stable benthic substrate (Fig. 1a). The plant is most common in rivers with an open canopy and a cobble or bedrock substrate, but it can also be found in smaller tributaries in locations with abundant light and perennial flow (e.g., waterfalls and cascades). *Podostemum ceratophyllum*, henceforth referred to as *Podostemum* (except where inclusion of the specific epithet provides needed clarity) can cover vast areas of the streambed and provides habitat, and potentially food, for a diverse group of aquatic organisms. *Podostemum* may also influence nutrient and carbon dynamics in the swift-flowing rivers where it occurs (Fig. 2).

Dayton (1972) used the term “foundation species” to describe an organism that strongly influences community structure and function. Later Ellison et al. (2005)

employed the foundation species concept to illustrate how the loss of certain tree species altered the local environment and important ecosystem processes like decomposition, nutrient flux, carbon sequestration and energy flow. Similarly, we propose that *Podostemum* can be considered a foundation species based on the plant's extensive geographic range and substantial influence on ecosystem processes and benthic community structure (Nelson and Scott, 1962; Everitt and Burkholder, 1991; Grubaugh and Wallace, 1995; Hutchens et al., 2004). *Podostemum* is morphologically and ecologically similar to riverine bryophytes, which also grow attached to stable substrates, provide substantial habitat for macroinvertebrates and epiphytic biofilms, and increase retention of organic matter and stream metabolism (Stream Bryophyte Group, 1999; Wood et al., 2016). However, we hypothesize that *Podostemum* has a stronger influence on ecosystem processes than bryophytes because it grows more quickly and in a broader range of light conditions, and sustains higher grazing pressure (Parker et al., 2007).

Podostemum is also of interest because the plant appears to be declining across much of its native range. Local extinction or substantial decline of *Podostemum* has been documented in several northern rivers including the Cocheco River near Dover, New Hampshire, the West River near Jamaica, Vermont (Philbrick and Crow, 1983), tributaries of the Roanoke River in Virginia (Connelly et al., 1999), several rivers in Pennsylvania (Munch, 1993) and possibly throughout much of the eastern Piedmont. The species is listed as Endangered, Historical, a Species of Concern or Threatened in many northern States (USDA, 2014). Decline and extirpation have been attributed to sedimentation, dewatering, inundation by water impoundment, and unspecified pollutants from industry, mining operations and urban runoff (Adams et al., 1973; Munch, 1993;

Connelly et al., 1999). However, neither the underlying factors nor the ecological significance of changes in *Podostemum* abundance have been extensively investigated.

This review provides a synopsis of the biology and ecology of *Podostemum* and identifies research needed to understand the causes and consequences of changes in abundance of the plant across its native range. We review reports describing *Podostemum* occurrence, important life history traits, and its role as a foundation species in eastern North American rivers (Table 1). We then hypothesize how *Podostemum* will likely respond to future environmental change, and how changes in *Podostemum* occurrence will likely affect river ecosystems.

Distribution and biology of *Podostemum ceratophyllum*

Biogeography

The family Podostemaceae Rich. ex C. Agardh is the largest family of strictly aquatic flowering plants in the world (Philbrick and Novelo, 1995; Philbrick and Novelo, 2004). These plants possess distinctive morphological adaptions including specialized root structures and long, thin durable leaves well-adapted to their swift-water habitat (van Steenis, 1981). North, Central, and South America contain about 60% of the species in the family, with the remaining species distributed throughout Africa, Madagascar, and Southeast Asia (Philbrick and Alejandro, 1995). Recent investigations have concluded that the genus *Podostemum* is restricted to the New World (Philbrick and Novelo, 2004), with the greatest species diversity occurring in South America, mainly in Brazil. South American Podostemaceae taxonomy remains uncertain (Philbrick et al., 2010) and ecological studies on these species are sparse. Mexico is reported to have four genera (Marathrum, Oserya, *Podostemum*, Tristicha) with higher diversity in the Pacific coast slopes compared with Atlantic slopes (Novelo and Philbrick, 1997; Tippery et al., 2011). Altogether, the Americas are thought to contain about 135 species of Podostemaceae with only a single species, *Podostemum ceratophyllum*, known from the continental U.S.A. and Canada (Graham and Wood, 1975; Philbrick et al., 2010; Tippery et al., 2011).

Podostemum ceratophyllum's native range is confined to montane and piedmont regions of the eastern United States and Canada, ranging from Georgia to Ontario, with scattered populations westward as far as Arkansas, Oklahoma, Minnesota and North Dakota, and disjunct populations in Honduras and the Dominican Republic (Philbrick and Crow, 1983; Philbrick and Novelo, 2004). Reduced genetic variation (based on nucleotide markers and isozymes) in populations north of North Carolina indicates range expansion northward following the last glacial-maximum from refugia several hundred km south of the glacial boundary (Philbrick and Crow 1992; Fehrmann et al. 2012).

Morphology

Two of the earliest papers about *Podostemum* detailed the structure of the plant's vegetative and reproductive organs (Warming, 1881, 1882). *Podostemum* follows the Root-Shoot model with the presence of distinct roots, stems (shoots) and leaves (Rutishauser et al., 2003). The stems may be heavily cutinized (hardened), appearing dark green to black, often in stark contrast to its leaves, which can be a vibrant green. Cutinization can result from damage to the stem (Hammond, 1937), and heavily cutinized stems may correlate with abrasion from suspended sediments in swift current. New growth is often a luxuriant green in spring and summer, while in the winter leaves often are completely senesced or take on a deep reddish color (Hammond, 1937). The red coloration is caused by an increase in the light-absorbing pigment anthocyanin, which reduces tissue damage from UV light but may have other functions. Production of anthocyanin is a common stress response in plants and has also been linked to nutrient imbalance (Marschner, 1986). Supportive of this conclusion, Munch (1993) only found *Podostemum* exhibiting the red coloration in surface water that had a total nitrate-N to total phosphorous ratio of more than 18:1.

The roots of *Podostemum* attach to stable substrates (rock, wood, and other debris) with distinct root hairs called haptera (Rutishauser et al., 2003). While the root hairs were once thought to exude a sticky substance that attached the plant to rocks, a study of Old World species of Podostemaceae proposed that attachment is facilitated by a film of cyanobacteria (Jäger-Zürn and Grubert, 2000). The nature of this relationship is not understood, and has not been investigated in *P. ceratophyllum*. Aside from the basic root-stem-leave structure, *Podostemum* is highly variable in appearance (Fig. 3). Four varieties have been described based on this variation (van Royen, 1951) but these varieties have been condensed into one species with highly plastic morphology (Philbrick and Novelo, 2004). Podostemum can have long leaves (4–20 cm) in the form once recognized as *P. ceratophyllum* var. *ceratophyllum*, or shorter leaves that are densely clustered at the end of the stem, giving the plant a distinctly bristly appearance (in the form once recognized as *P. ceratophyllum* var. circumvallatum). Hammond (1937) notes that these different forms can grow side by side but that plants in a given colony are generally uniform in size and structure. We hypothesize that specific aspects of the habitat such as flow velocity, herbivory, or both may exert a large influence on growth form.

Reproduction

Flower buds open as water levels decline and the plant is exposed above the water surface (Philbrick, 1984). Flowers emerge from an enclosed spanthellae, and mature flowers (Fig. 1b) have obvious anthers subtended by an enlarged ovary with two stigma (Philbrick, 1984). Pollination is most likely facilitated by wind or insects, but not water, and pre-anthesis cleistogamy (pollination before the flower opens) has also been reported (Philbrick, 1984). After pollination maturation of the seed capsule is reported to take 2–3 weeks (Philbrick, 1984) and seed capsules may appear mature while still developing (Philbrick and Novelo, 1995). The seeds are small and the seed coat produces a sticky mucilaginous coating when wetted, allowing seeds to stick to suitable substratum. While pollination and seed dispersal mechanisms have not been intensively investigated (Philbrick, 1984), gene flow between populations appears erratic (Fehrmann et al., 2012) and seed dispersal is presumably facilitated by migrating wildlife (birds & large mammals), while long distance dispersal is probably limited to avian vectors (Philbrick and Crow, 1992).

Philbrick and Novelo (1994) propose that Podostemads use the type 1 seed germination strategy, first proposed by Thompson and Grime (1979), where seeds germinate soon after being released from the capsule. Indeed, the seeds lack an endosperm, show no need for cold stratification or dormancy, and seem unlikely to persist for years before germination (Philbrick, 1984). Additionally, asexual reproduction is facilitated by root fragmentation, where detached root segments can reattach to rocks over time (Philbrick et al., 2015). For additional details about morphology, development and reproduction refer to (Graham and Wood, 1975; Philbrick, 1984; Philbrick and Alejandro Novelo, 1997; Rutishauser, 1997; Rutishauser et al., 2003; Philbrick and Novelo, 2004).

Physiology

Information about oxygen and carbon dioxide uptake rate and almost all other physiological responses of Podostemaceae is limited. Unlike most other aquatic plants which can utilize bicarbonate in addition to dissolved carbon dioxide, *Podostemum* may only be able to absorb dissolved carbon dioxide from the water column (Pannier, 1960; Hill and Webster, 1984) − a trait shared with bryophytes. Thus, a study on the New River attributed reduced ^{14}C uptake at soft-water sites to reduced availability of free CO_2 compared to hard-water sites (Hill and Webster, 1984). While the respiration rate of *Podostemum* has not been investigated, the neo- and paleotropical taxon (Tristicha trifaria (Bory ex Willd.) Spreng.) is reported to have an ability to absorb oxygen at an extremely high rate (14 mg O_2 g dry wt^{-1} h^{-1}) in oxygen-saturated water (Pannier, 1960).

***Podostemum* as a foundation species**

High biomass and productivity

Several studies have indicated that *Podostemum* is highly productive and capable of obtaining large standing stock biomass, although variation among locations, seasons and years may be substantial. Hill and Webster (1983) estimated that *Podostemum* contributed 1154 T ash free dry weight (AFDM) yr^{-1} to their New River, Virginia study area, approximately 80% of the total macrophyte contribution. *Podostemum* production was 10 times that of periphyton on an aerial basis and the ratio of production to biomass (P/B) was as high as 4 (most aquatic macrophytes are closer to 2; Hill and Webster 1984). The authors interpreted this high production relative to biomass as indicative of substantial biomass loss to scouring (Hill and Webster, 1984), although the potential influence of herbivory was not measured. Not surprisingly, measures of productivity have varied substantially, likely reflecting the influences of flow, water chemistry and location within the channel. For example, estimated productivity spanned 3 orders of magnitude (0.05 g C m^{-2} d^{-1} to 1.08 g C m^{-2} d^{-1}) on the New River and Watauga River (Tennessee) (Hill and Webster, 1984).

Biomass measurements have also varied widely, likely reflecting multiple influences. Rodgers et al. (1983) reported a seasonal maximum biomass between 22 and 98 g AFDW m^{-2} on the New River and Watauga River, in contrast to substantially higher mean monthly standing stocks (between 386 and 587 g AFDM m^{-2}, to a maximum of just over 1000 g AFDM m^{-2} in November) on the Middle Oconee River, Georgia, (Grubaugh and Wallace 1995). Biomass measurements at the same Middle Oconee River site during a prolonged drought were an order of magnitude lower (Pahl, 2009).

Influences on benthic biota

For almost 100 years, ecologists have known that macroinvertebrates utilize the habitat produced by *Podostemum* (Hammond, 1937) and more recent studies have shown strong correlations between *Podostemum* and abundances of some riverine biota (Hutchens et al., 2004; Argentina et al., 2010b). A study in the Little Tennessee River, North Carolina, found *Podostemum* enhanced the surface area of macroinvertebrate habitat on bedrock by at least 3–4 times, and that removal of *Podostemum* reduced macroinvertebrate biomass by over 90% and abundance by almost 88% (Hutchens et al., 2004). A wide diversity of macroinvertebrates are associated with *Podostemum*. Rocks colonized by *Podostemum* in the Middle Oconee River contained at least thirty-four genera of aquatic insects (plus an additional 13 taxa only identified to family level or the order Hemiptera) representing all major aquatic insect orders, as well as Cnidaria, Tubellaria, Mollusca, Annelida, Hydracarina, Cladocera, and Copepoda (Nelson and Scott, 1962; Grubaugh and Wallace, 1995).

Podostemum may particularly enhance habitat availability for filter-feeding insects by providing points of attachment with access to swiftly-flowing water. The silk nets of hydropsychid caddisfly larvae are commonly observed in *Podostemum* mats (pers. obs. J.W.), and the plant is reported to support significantly higher abundances of hydropsychids (Tinsley, 2012) than bare rock. Similarly, densities of the filter-feeding

Etowah caddisfly, Brachycentrus etowahensis Wallace, have been positively correlated with *Podostemum* (Willats, 1998; Duncan, 2008). The plant also appears to be a preferred habitat for filtering black fly larvae (Simulium), with measured densities of 4.2–4.5 individuals per square cm of *Podostemum* stem, among the highest densities recorded for the 54 plant taxa examined in a mesocosom study (Hudson and Hays, 1975). Furthermore, Hutchens et al. (2004) report that filterers were the best represented macroinvertebrate functional feeding group (FFG) in *Podostemum* by biomass.

Podostemum may also attract other FFGs because the plant traps organic matter and provides a substrate for epiphytic overgrowth of diatoms and other algae (Fig. 4). Thus, insects that feed by scraping periphyton (scrapers) or by collecting fine detrital particles (collector-gatherers) can be the most abundant FFGs associated with *Podostemum* (Hutchens et al., 2004; Grubaugh and Wallace, 1995). Similarly, snails, which are among the most endemic and threatened riverine invertebrates in eastern rivers (Johnson et al., 2013), are frequently observed grazing on *Podostemum*. In a study on the Yellow River, Georgia, Krieger and Burbanck (1976) found that *Podostemum* created the optimum habitat for the freshwater snail Pleurocera catenaria (Say) and other investigators have concluded that the presence of *Podostemum* and stable benthic substrates were the most important factors in predicting pleurocerid (especially Elimia spp.) snail distribution (Mulholland and Lenat, 1992; citing Krieger and Burbanck’s 1976 study).

Associations between fish and *Podostemum* have been noted (Freeman and Freeman, 1994; Connelly et al., 1999; Skelton and Albanese, 2006; Argentina et al., 2010b; Ashton and Layzer, 2010) but a general lack of experimental research prohibits definitive conclusions. Short-term experimental manipulations of *Podostemum* in the Conasauga River, Georgia, by Argentina et al. (2010b) showed declines or increases in local benthic fish densities where *Podostemum* was reduced or augmented, respectively. The increased habitat complexity provided by *Podostemum* may benefit fishes by increasing densities of insect prey and by providing shelter from larger predators. However, species associations with *Podostemum* at landscape-scales can be difficult to untangle from other basin wide stressors that negatively influence species (Argentina et al., 2010a).

Podostemum may influence aquatic flora other than epiphytic algae, although we know of only a single study of competition with other submerged macrophytes. Everitt and Burkholder (1991) conclude that *Podostemum* uses a strategy of niche preemption to maintain habitat and prevent invasion by other species such as the red alga Lemanea australise Atkinson. In cool temperature months Lemanea and *Podostemum* are co-dominant, however, *Podostemum* grows most readily in the spring and summer months wherever light permits. *Podostemum* then dominates during the warm season but loses ground to other species in the fall and winter (Everitt and Burkholder, 1991).

Contributions to detrital and autotrophic foodwebs

Podostemum contributes to foodwebs directly and indirectly. *Podostemum* may indirectly enhance organic detritus retention by trapping particles entrained in the water column and accumulating fine sediments around the base of the plant. Stems and leaves directly contribute to detrital pathways (Nelson and Scott, 1962) and may senesce at biologically important times, i.e. late fall and early winter (Hill and Webster, 1982). Indeed, studies of seasonal changes in *Podostemum* biomass generally indicate that biomass is highest in early fall then declines as the plant senesces sensitive tissue (Rodgers et al., 1983; Grubaugh and Wallace, 1995) (but see Nelson and Scott, 1962). Seasonal changes in biomass may also be related to minimum water temperature, light availability, or other biotic and abiotic factors.

The leaves and stems of *Podostemum* decompose relatively quickly and contribute to the detrital pool. Rodgers et al. (1983) report a breakdown rate (K) between 0.05 and 0.08 g g^{-1} d^{-1} (5–8% per day, depending on water temperate), and a 95% loss interval of 60 days in the New and Watauga rivers. Hill and Webster (1982) found a similar breakdown rate of 0.04 g g^{-1} d^{-1}, with a 95% loss interval of 81 days on the New River. These breakdown rates are an order of magnitude (or more) greater than the rate for allochthonous material, where $K < 0.02$ (Petersen and Cummins, 1974; Rodgers et al., 1983; Kominoski et al., 2007), indicating that carbon stored in Podostemum tissues is more rapidly recycled through the ecosystem compared to terrestrially-derived leaf litter. The importance of Podostemum's direct contribution to the food web is uncertain. Herbivory by Canada geese (Branta canadensis (Linnaeus)) and White Tubercled crayfish (Procambarus spiculifer (Le Conte)) has been reported (Parker et al., 2007), and Weberg et al. (2015) raised the possibility of consumption by introduced triploid Grass Carp (Ctenopharyngodon idella (Valenciennes)) in the New River. We and others have observed aquatic turtles (e.g., Pseudemys spp; Fahey (1987) in Aresco and Dobie (2000)), Whitetail deer (Odocoileus virginianus (Zimmermann)) and Beaver (Castor canadensis Kuhl) grazing on Podostemum (pers. obs. M.F.). However, quantitative studies of herbivory rates or the relative contribution of Podostemum to aquatic primary consumers are lacking. The nutritional value of Podostemum is not well known. At present, only two published studies are known to have reported the elemental composition of Podostemum tissue. A study conducted in Pennsylvania rivers by Adams and coauthors (1973) reported concentrations of P, K, Ca, Fe, Mg, B, Cu, Mn, Al, Zn, and Na, while Heisey and Damman (1982) investigated copper and lead accumulation in aquatic plants including Podostemum downstream of industrial outfall into the Shetucket and Natchaug Rivers, CT. Adams and coauthors (1973) report that Podostemum was 0.25% P by dry mass, while K, Ca, and Mg were 1.63, 1.38 and 0.24% respectively. Unpublished data (J.W.) indicate that on average *Podostemum* is 2.7% nitrogen and 36.4% carbon, with a molar carbon:nitrogen ratio of 16.2:1 (Unpublished J.W.), similar to other submerged freshwater plants (Bakker et al., 2016). While only limited inferences can be made from these studies, *Podostemum* may be a source of ecologically important elements for grazing organisms, especially nitrogen, phosphorus, calcium, and trace metals.

Environmental stressors

Sedimentation and flow alteration

Fast-flowing water, stable benthic substrate and sufficient light are the major factors consistently correlated with the occurrence of *Podostemum* (Everitt and Burkholder, 1991; Connelly et al., 1999; Argentina et al., 2010a; Duncan et al., 2011). *Podostemum* commonly occurs on coarse sediments of sandstone, shale, or granite (but rarely limestone (Meijer, 1976)), as well as other submerged substrates including wood, tires, plastics, aluminum, ceramics and other debris (per. obs. J.W.). Excessive sedimentation either through increased sediment load in the river or reduced sediment transport capacity, has been cited as a reason for *Podostemum* decline. For example, Connelly et al. (1999) cite sedimentation and streambed instability as possible reasons for declines in *Podostemum* abundance in the Roanoke River, Virginia. Similarly, Grubaugh and Wallace (1995) attribute an increase in *Podostemum* biomass on shoals in the Middle Oconee River to declining agriculture, and presumably sedimentation, in the watershed.

Hydrologic alteration can reduce *Podostemum* cover by decreasing wetted instream habitat and influencing flow velocity. Substantial dieback of *Podostemum* has been documented during a severe drought in the southeast U.S. that resulted in extended exposure of *Podostemum* above the waterline (Pahl, 2009), and flow manipulations downstream from a reservoir are reported to have resulted in the extirpation of a population of *Podostemum* in the West River at Jamaica, VT (Countryman, 1978). Although *Podostemum* has subsequently been found at other locations in the West River (Zika and Thompson, Zika and Thompson, 1986) (pers. obs. J.W.), flow regulation may influence population dynamics for many kilometers downstream of the source of regulation. Periodic exposure to drying and substantial reductions in water velocity may be mechanisms by which flow regulation reduces *Podostemum* cover and biomass. Supportive of this idea, Everitt and Burkholder (1991) report that *Podostemum* in their study could not tolerate even short periods of desiccation. Furthermore, slack water behind impoundments may permanently extirpate populations. For example, two populations of *Podostemum* in New Brunswick, Canada are reported to have been inundated to a depth that prevented persistence (Philbrick and Crow, 1983). Collectively, these studies support a conceptual model that includes flow as an important ecological variable, with diminution in water level and flow velocity potentially reducing *Podostemum* occurrence and biomass.

Influences of temperature and water chemistry

The influence of water temperature and dissolved gas concentration on *Podostemum* have not been evaluated but may be important given predictions of increasing water temperature with climate change (Ficke et al., 2007) and watershed urbanization (Wenger et al., 2009). Munch (1993) reports finding *Podostemum* in rivers in PA between 0 and 30ºC, but some southern populations likely experience water temperatures routinely exceeding 30ºC during summer months. Restricted CO_2 availability, such as in slow moving water or with dense epiphytic algal overgrowth (Fig. 4) may also reduce *Podostemum* growth rate and accrual of biomass. Furthermore, Hill and Webster (1984) hypothesize that differences in water hardness are responsible for a two-fold difference in biomass between study sites on the New River, NC (see Section 2.3. Physiology). Investigations of variation in stable carbon ratios could elucidate differences in CO_2 availability among habitats. Ziegler and Hertel (2007) argue that observed variation of $\delta^{13}C$ in *Podostemum* leaf tissue reflects variation in boundary layer “diffusional resistance” because the plant appears to preferentially utilize the $\delta^{12}C$ isotope of CO_2 compared to the heavier $\delta^{13}C$ isotope.

Tolerance to environmental pollutants

Meijer (1976) reports that *Podostemum* is generally found in clear streams with good aeration and sufficient light, and speculates that *Podostemum* might be useful as an indicator of clean water. However, Philbrick and Crow (1983) note that several populations have been found in polluted water, including in the Mousam River in Kennebunk Maine, where the river is polluted by domestic sewage. Similarly, a study of nutrient levels in Mexican rivers containing Podostemaceae documented occurrences of *Podostemum ricciiforme* (Liebm.) P. Royen at sites ranging from ultra-oligotrophic to hypertrophic (Quiroz et al., 1997), showing that certain species of *Podostemum* can tolerate high nutrient levels or other forms of water pollution. Nonetheless, road salts (Jackson and Jobbagy, 2005; Kaushal et al., 2005), deicers (Fay and Shi, 2012) and other aspect of urbanization (Walsh et al., 2005; Chin, 2006) may constitute significant stressors to *Podostemum*.

Response to climate and land use change

A warming climate may facilitate the spread of *Podostemum* northward, continuing historical range expansion patterns (Philbrick and Crow, 1992; Fehrmann et al., 2012), Climate change may also exacerbate stresses already experienced by the plant, such as increased flow alteration, increased water temperature and increased sedimentation resulting from intense precipitation events. Accurately predicting the future distribution of *Podostemum* is complicated by the complexity of interacting stressors and the differing scales of controls on species migration (Pearson and Dawson, 2003).

Investigations into how *Podostemum* responds to changes in land use are needed in light of the rapid landscape changes occurring in many parts of this species' range. Isotopic nitrogen signature ($\delta^{15}N$) has been used to investigate the impacts of urbanization and land use on microbial biofilms (Kaushal et al., 2006), fish (Northington and Hershey, 2006) and riparian plants (Kohzu et al., 2008), and could be useful in assessing land use impacts on *Podostemum*, as well as measuring *Podostemum's* role in food chains (Cabana and Rasmussen, 1996). Urban runoff can also contain high concentrations of metals (Davis et al., 2001; Sörme and Lagerkvist, 2002; Rule et al., 2006) available for uptake by primary producers. If *Podostemum* bioaccumulates metals then herbivory would facilitate the transfer of water column pollutants into higher trophic levels, with possible ecological and human health concerns.

Synthesis: causes and consequences of changes in *Podostemum* abundance

Known and hypothesized influences on *Podostemum* biomass include several interacting factors: severity and duration of low-flow periods, water velocity, herbivory, sedimentation, light and nutrient availability, and substrate stability (Fig. 5). Previous studies have shown that prolonged reductions in discharge reduce plant biomass (Nelson and Scott, 1962; Pahl, 2009), thus we hypothesize that high-velocity habitats support higher *Podostemum* biomass by limiting herbivory by consumers unable to hold position in swift currents, and by reducing sedimentation and algal buildup that, in turn, reduce light availability. Discharge and water velocity may also influence water temperature, conductivity and dissolved gases (CO_2 and O_2) but the direct effects of these variables on *Podostemum* are not well known (Fig. 5).

Understanding effects of more frequent and prolonged periods of low-flow may be essential to predicting persistence of *Podostemum* in areas experiencing declining rainfall or increased water diversions for human uses. We expect that *Podostemum* responds differently to low-flow periods than other aquatic plants, and uniquely different from the macrophyte model proposed by Suren and Riis (2010). Specifically, we hypothesize that *Podostemum* biomass declines as rivers move into seasonal low flow periods, whereas rooted macrophytes exhibit a general increase in biomass with low flow conditions, and bryophytes maintain relatively stable biomass through the river's normal range of flow (Fig. 6). We also hypothesize that *Podostemum* biomass rapidly declines as the duration of low-flow conditions increases in response to increased herbivory, epiphytic overgrowth, and risk of drying, with the effect exacerbated by other water quality stressors.

One challenge for understanding *Podostemum* response to stressors is that field measurements may differ among local habitat types. Rivers in the eastern montane and piedmont regions are frequently characterized by alternating shoal (cascade, riffle, rapid) and pool habitats, and we hypothesize that these two habitats expose *Podostemum* to differing stressors as a result of differences in flow velocity and water depth. We speculate that biomass in pool habitats is strongly controlled by herbivory pressure, light availability and sedimentation rate, whereas shoal habitats provide increased protection from herbivory and sedimentation but expose the plant to increased risk of drying during periods of low flow.

We conclude that evidence supports the notion that *Podostemum* acts as a foundation species in many eastern rivers, removing nutrients from the water column, accumulating substantial benthic biomass, and shuttling resources into the food chain, in addition to providing habitat for a diverse flora and fauna. Loss of the plant from rivers where it presently occurs could thus reduce: 1) invertebrate biomass and resources for aquatic and terrestrial insectivores; 2) retention of nutrients in the benthos, influencing carbon balance and nutrient spiraling length; 3) retention of organic matter and resources for aquatic detritivores; 4) stream bed stability and complexity, increasing the severity of flood scour on the benthos; and, 5) export of autochthonous organic matter and thus resources available downstream. However, much of what we know about the ecology of *Podostemum* derives from studies in the southern portion of the species range (Table 1) and regional differences in genetics may influence responses to stressors. Information on responses of the plant to environmental changes throughout its range is essential to understanding how to conserve or restore populations. Conservation efforts would also benefit from better documentation of *Podostemum* populations, a long recognized deficiency in our understanding of the plant (Muenscher and Maguire, 1931). As pressures on freshwater resources increase, conserving *Podostemum* appears crucial for preserving and improving the health and vitality of many eastern North American Rivers.

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Table 3.1. Papers on the ecology of *Podostemum ceratophyllum* Michx. or that contain ecologically relevant information on the ecology the plant.

Topic	Foci	Author	Study Location
Macroinvertebrates	secondary production and community composition	Nelson and Scott 1962	GA, Middle Oconee River
	habitat preference and density of black flies (*Simulium decorum* Walker)	Hudson and Hays 1975	AL, Alabama Agricultural Experimental Station at Auburn University, Farm Pond no 1. artificial channel
	habitat preference of riverine snails (*Oxytrema (=Goniobasis) suturalis* Haldeman)	Kreiger and Burbanck 1976	GA, Yellow River
	secondary production and community composition	Grubaugh and Wallace 1995	GA, Middle Oconee River
	secondary production and impact of plant removal treatment	Hutchens et al. 2004	NC, Little Tennessee River
	habitat of the caddisfly (*Brachycentrus etowahensis* Wallace)	Duncan PhD Dissertation 2008	GA, Upper Etowah River
	dietary preference and habitat of Hydropsychid caddisflies	Tinsley BS Thesis 2012	KY, Upper Green River
Macrophyte community dynamics and regrowth	interspecific competition between benthic autotrophic	Everitt and Burkholder 1991	NC, Main stem and Cedar Fork of the Little River
	regrowth from root fragments	Philbrick et al. 2015	CT, Pootatuck River
Fishes	habitat use by Riverweed Darter *(Etheostoma podostemone* Jordan & Jenkins)	Connelly et al. 1999	VA, North and South Fork of Roanoke River
	habitat preference of riverine fish and influence of *Podostemum*	Argentina et al. 2010	GA & TN, Conasauga River
	habitat preference of the Snail Darter (*Percina tanasi* Etnier)	Ashton and Lazer 2010	TN, French Broad and Hiwassee Rivers
Flow	flow alteration and plant recovery	Pahl MS thesis 2009	GA, Middle Oconee River, Honeycutt Creek
Herbivory	consumption by River Cooter (*Pseudemys concinna* (Le Conte))	Fahey 1987 in Aresco and Dobie 2000	AL, Tallapoosa River

	consumption by Canada geese, crayfish, & amphipods	Parker 2007	GA, Chattahoochee River and in the laboratory
	consumption by triploid Grass Carp (*Ctenopharyngodon idella* (Valenciennes))	Weberg et al. 2015	VA, Upper New River
Habitat	influence of land use, light, and substrate size	Argentina et al. 2010	GA & TN, Conasauga River
	influence of channel morphology and substrate size	Duncan et al. 2011	GA, Upper Etowah River
Decomposition rate	*P. ceratophyllum* breakdown rate	Hill and Webster 1982	NC & VA New River
		Rodgers et al. 1983	TN, Watauga & VA, New Rivers
Productivity	*P. ceratophyllum* production	Hill and Webster 1984	NC & VA New Rivers
Elemental	plant elemental composition	Adams et al 1973	DE, Susquehanna
	copper and lead bioaccumulation	Heisey and Damman 1982	CT, Natchaug, Willimantic and Shetucket Rivers
Biogeography	species distribution	Philbrick 1983	Eastern US, Arkansas, Honduras, Dominican Republic
	isozyme variation	Philbrick and Crow 1992	Eastern US
	interspecific nucleotide diversity	Fehrmann et al 2012	Eastern US, Arkansas and Honduras
Other	cyanobacterial symbiotic relationship	Jager-Zurn and Grubert 2000	herbarium samples (Old World species only)
	carbon Isotope fractionation	Ziegler and Hertel 2007	herbarium samples

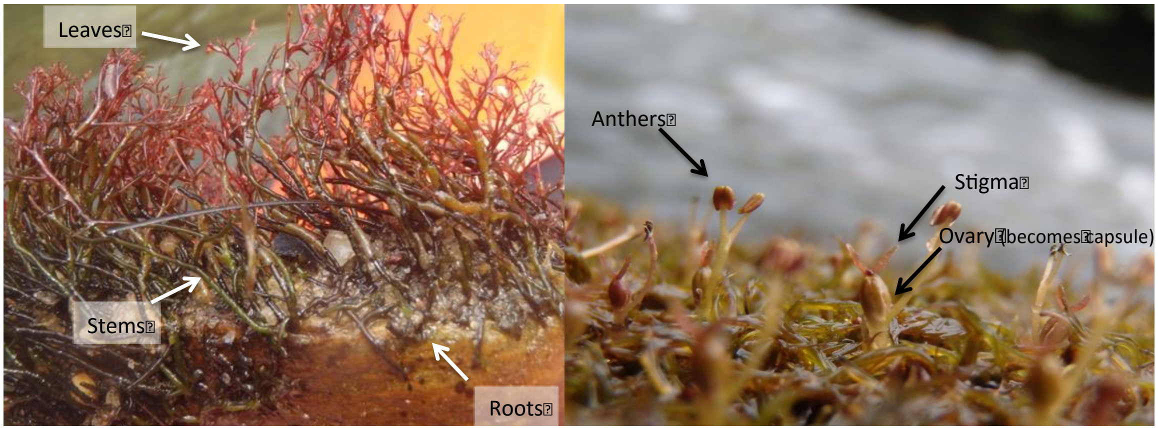

Figure 3.1. (a) *Podostemum ceratophyllum* grows submerged, attached directly to rocks in fast flowing eastern rivers. Stems and leaves can be green, black and red and the leaves are deeply dichotomously lobbed. Roots are also green, black and red and attach the plant to the rock with structures called haptera. (b) Flowers emerge as water levels expose the plant above the water's surface. Flowers are small with reduced petals and prominent anthers above the stigma and ovary. Photo by J. Wood. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

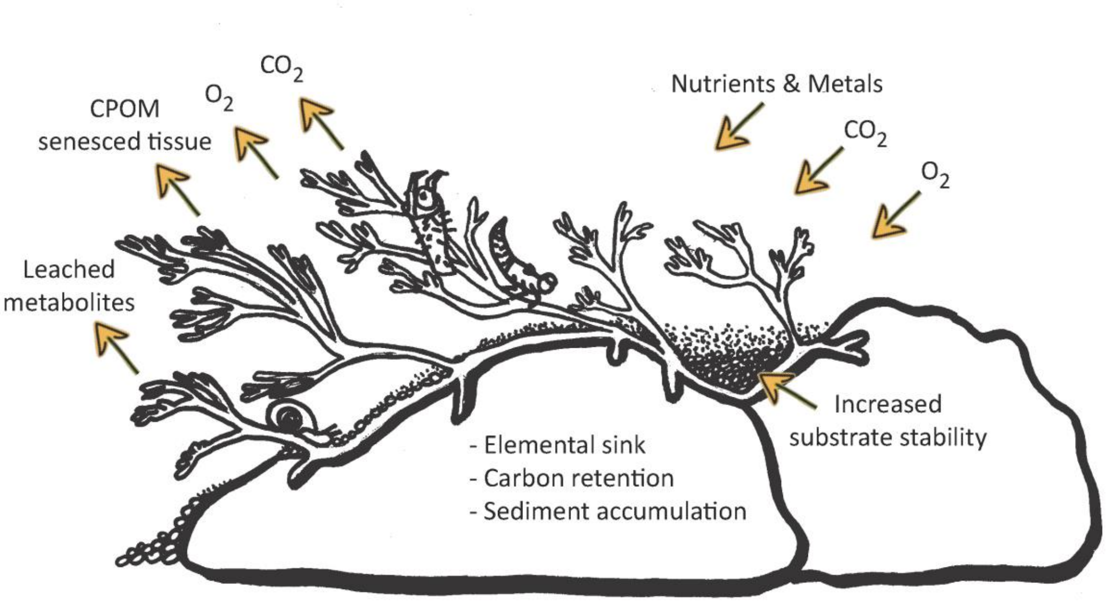

Figure 3.2. A diagram illustrating *Podostemum ceratophyllum*'s interactions with the benthic environment. *Podostemum* provides structure and increases habitat complexity over bare rock, which attracts riverine biota. *Podostemum* influences elemental cycling through: retention of detrital material in plant colonies; assimilation of elements from the water column into plant tissue; and leaching of metabolites into the water column. *Podostemum* also increases substrate stability by binding gravels and cobbles together

Figure 3.3. Examples of the morphological variation, from extended, narrow leaves to short, broader leaves, common in *Podostemum ceratophyllum.* These stems were collected on the same day and in close proximity to each other. Small squares in the background are 1 mm × 1 mm. Photo by J. Wood.

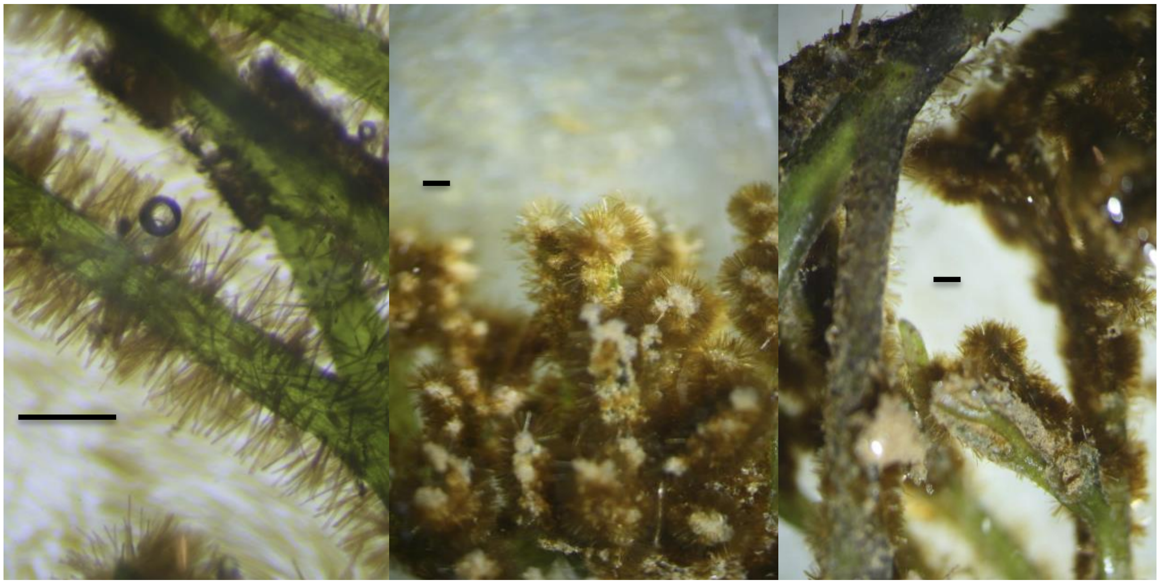

Figure 3.4. Magnified images of *Podostemum ceratophyllum* stems with diatom (*Synedra ulna* c.f.) overgrowth. *Synedra ulna* cell length approximately 0.3 mm, scale bar approximately 5 mm in each picture. Fine sediments have accumulated between diatom cells and have encapsulated *Podostemum*'s stems and leaves in a nearly complete overcoating. Photos taken by J. Wood on November 11, 2013 from samples collected on a bedrock shoal on the Middle Oconee River, at Ben Burton Park, Athens, GA.

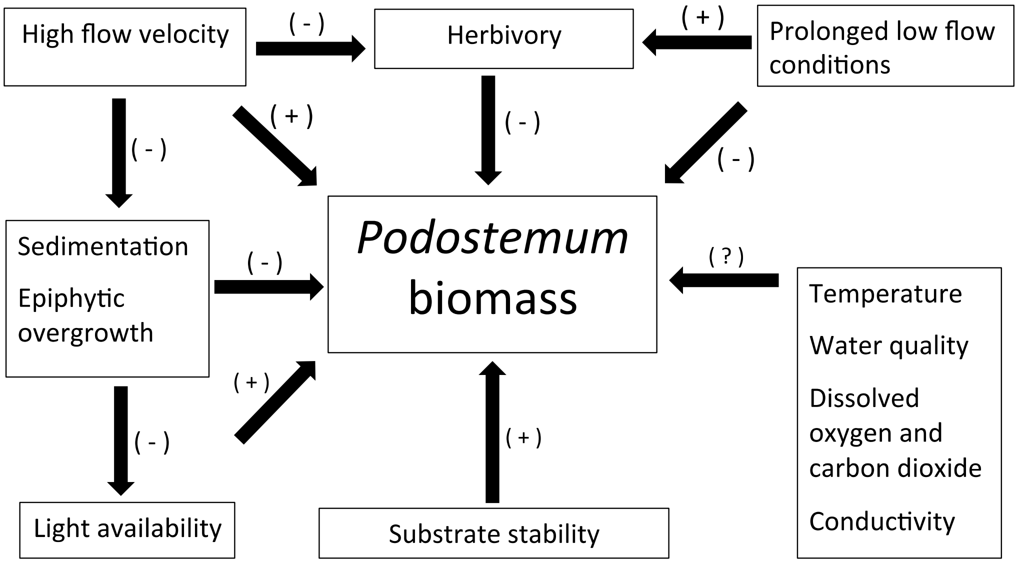

Figure 3.5. Hypothesized relationships between *Podostemum ceratophyllum* and the dominant environmental variables of the habitat. Arrows indicate the directional nature of the relationship; positive associations are shown as (+) and negative associations are shown as (−).

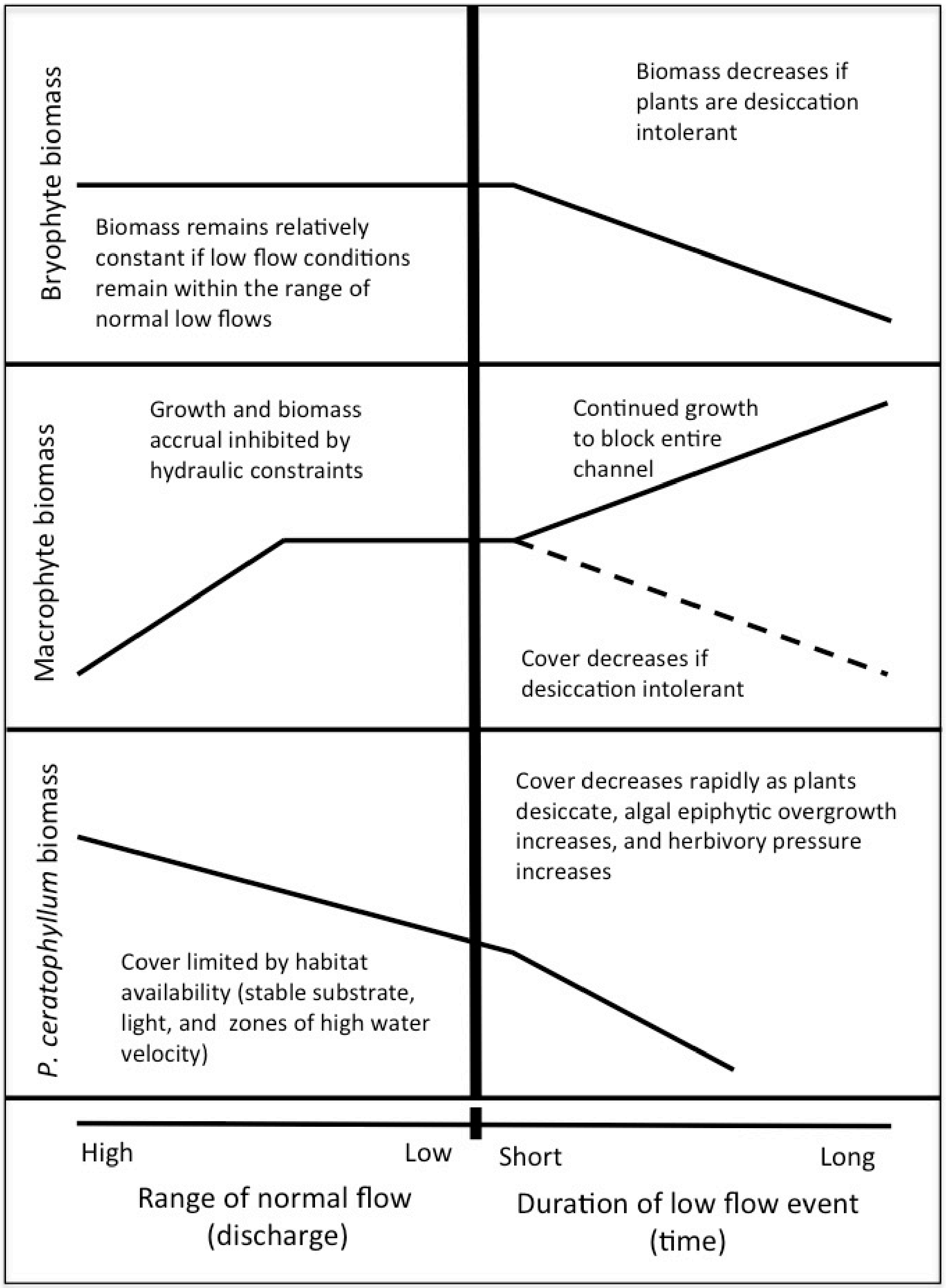

Figure 3.6 Hypothesized relationships and comparisons between flow (discharge) and the duration of low flow events (time) for bryophyte, macrophyte, and *Podostemum ceratophyllum* Michx. biomass (modified from Suren and Riis, 2010).

CHAPTER 4

HIGH WATER VELOCITY MEDIATES HERBIVORY PRESSURE ON *PODOSTEMUM CERATOPHYLLUM* MICHX. BIOMASS AND RESOURCE AVAILABILITY TO BENTHIC CONSUMERS[1]

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[1] Wood, J. L, M. C. Freeman, and J. W. Skaggs. To be submitted to Freshwater Biology

Abstract

Species responses to environmental variables influence trophic interactions, which can affect ecosystem structure and function by altering storage and transfer of basal resources through ecosystems. In freshwater ecosystems, water velocity is a defining characteristic of riverine habitat but water velocity is influenced by stochastic events and through management decisions. *Podostemum ceratophyllum* is foundation species of eastern North American rivers and grows attached to stable substrates in high water-velocity habitats, and positively affects benthic habitat complexity, macroinvertebrate biomass, and fish abundance. Although *Podostemum ceratophyllum* is eaten by a wide variety of consumers, the interacting effects of herbivory and water velocity on the plant are unknown. We examined the effects of water velocity and herbivory experimentally by utilizing consumer exclosures to quantify herbivory pressure, and by altering local water velocity to investigate interactions between water velocity and change in plant biomass. We estimated that 85% (67 – 98%; 95% credible interval) of the daily stem growth (0.026 cm cm^{-1} day^{-1}) in *Podostemum* was consumed during a 77-d paired consumer access versus exclosure experiment. We also found evidence that water velocity significantly influenced *Podostemum* biomass by reducing herbivory pressure in high water-velocity habitats. Biomass loss occurred rapidly when the regulating effect of water velocity was reduced and consumers gained access to the plant. We conclude that fluctuations in water velocity modulate the accrual of *Podostemum* and the movement of *Podostemum*-derived materials through benthic food webs. High water velocity habitats may thus act as *Podostemum* accrual zones, whereas

low velocity habitats facilitate the movement of *Podostemum* production into food webs. This research has implications for estimating resource storage and flux in lotic food webs and illuminates a mechanism by which flow regulation and management may affect basal resources in rivers.

Introduction

Species responses to abiotic variables have been the foci of many preeminent ecological studies (Cowles, 1899; Gleason, 1926) because abiotic variables (e.g., light, temperature, nutrient available) exert biologically significant effects on the structure of ecological communities. In lotic systems, water velocity can exert a strong influence on species interactions (Doyle, 2006) but water velocity is also spatially variable within the river channel. Because lotic habitats are biologically diverse and dynamic, understanding how changes in water velocity may influence the movement of basal resources through the ecosystem is important for conservation of biodiversity and for predicting the effects of climate change and water management decisions on ecosystem function.

Macrophytes (aquatic plants) play important roles in aquatic ecosystems and are increasingly being recognized as enhancing benthic structural complexity and resource availability for aquatic fauna (Carpenter and Lodge, 1986; Bakker et al., 2016) and epiphytic algae (Tóth, 2013). Macrophytes also facilitate the movement of water-column nutrients into food webs, via the storage of nutrients in plant tissue and the subsequent release of nutrient through ingestion by consumers. Herbivory on freshwater macrophytes is widely documented (Carpenter and Lodge, 1986; Lodge, 1991; Newman, 1991), and studies examining trophic interactions of macrophytes in rivers indicate that water velocity can influence herbivory pressure (Doyle, 2006).

Lotic ecosystem structure and function are strongly influenced by intra-annual and inter-annual changes in discharge and water velocity (Poff et al., 2009), including effects of elevated discharge and velocity on primary producers as basal resources. High discharge events and moderate water velocities are generally thought to reduce standing stocks and increase export of basal resources. For example, Riis and Biggs (2003) found that macrophytes were rare in streams with more than 13 flood disturbance events per year, and the authors report a three-fold decrease in macrophyte biomass in streams with more than eight disturbance events a year, compared with streams without disturbance. Local water velocities above approximately 0.5 m s^{-1} have been found to decrease macrophyte biomass (Chambers et al., 1991) and reduce macrophyte diversity (Nilsson, 1987). Furthermore, periphyton can be similarly affected, with a nearly threefold decrease in algal biomass as disturbances events increase from 0 – 8 per year (Biggs, 1995), and reduced biomass at water velocities above 0.3 m s^{-1} (Biggs and Stokseth (1996) and 0.5 m s^{-1} (Horner and Welch (1981). Similarly, Hondzo and Wang (2002) found water velocities as low as 0.15 m s^{-1} can increase the removal of filamentous algal. Conversely, other experiments have found water velocity has little influence on periphyton biomass up to velocities of approximately 0.8 m s^{-1} (Horner et al., 1990). Collectively, these studies illustrate the potential for higher water velocities to depress macrophyte or algal biomass, either by preventing establishment or by removing material during high discharge events. These studies also raise compelling questions about the influence of water velocity on autotrophic biomass accrual and potential interactions with consumers.

Podostemum ceratophyllum Michx., henceforth called *Podostemum,* is a common submerged macrophyte in rivers in the montane and piedmont regions of eastern North America, with a range extending as far west as Arkansas and including disjunct populations in the Dominican Republic and Honduras (Philbrick and Crow, 1983). *Podostemum* grows attached to stable substrates and is thought to be restricted to swift-water habitats where ample light reaches the benthos. *Podostemum* has been described as a foundation species of eastern rivers (Wood and Freeeman, 2017) because the plant can cover large portions of the benthos, positively influences macroinvertebrate biomass and abundance (Grubaugh and Wallace, 1995; Hutchens et al., 2004) and occurrence of fishes (Connelly et al., 1999; Argentina et al., 2010), is consumed by a variety of vertebrate and invertebrate consumers (Parker et al., 2007; Weberg et al., 2015) and contributes substantial material to detrital food webs (Nelson and Scott, 1962). Furthermore, *Podostemum* biomass can vary widely, reaching upwards of 1000 g m^{-2} in some locations (Grubaugh and Wallace, 1995). The plant appears to be declining across much of its range, especially in northeastern North America (USDA, 2014), however causes of decline are unknown.

We sought to investigate how water velocity and herbivory interact to modulate storage and flux of *Podostemum* production into benthic food webs. Because *Podostemum* characteristically grows in swift water velocities, we would expect it to be less sensitive to velocity than periphyton and other macrophytes. We hypothesized that herbivory pressure strongly influences standing stocks of *Podostemum* biomass and that high velocity locations within the channel could reduce herbivory pressure on the plant thus increasing plant biomass. Specifically we asked the following questions: 1) Can water velocity regulate herbivory pressure on *Podostemum ceratophyllum*?, 2) Is there evidence that low-velocity conditions alone negatively impact *Podostemum*?, 3) Do large-bodied herbivores (e.g., geese, turtles, and crayfish) influence *Podostemum* biomass accrual, and by how much?

Methods

Study Location

We conducted several experiments on the interactions between water velocity and herbivory at three locations in two eastern piedmont rivers in the Altamaha River basin, Georgia, USA (Figure 1). Three studies were conducted on the Middle Oconee River near Athens, Georgia, a 6th order Piedmont river characterized by shifting sand substrates interspersed by bedrock outcroppings and cobble shoals (Nelson and Scott, 1962; Grubaugh and Wallace, 1995; Katz and Freeman, 2015). We used two shoal locations in the Middle Oconee River (Figure 1), one of which (Tallassee Shoals) was located directly downstream from a diversion dam for a hydropower raceway and municipal water withdrawal, whereas the other site (Ben Burton Park) was approximately 12 km downstream and primarily affected by the municipal water withdrawals. We repeated one set of experiments in the nearby Apalachee River (downstream from the US Hwy 78 bridge near Athens, GA) to confirm that our results were not unique to the Middle Oconee River.

Experimental Design

Three separate experiments were developed to explore relationships between *Podostemum*, herbivory, and water velocity. To answer the question “Can water velocity regulate herbivory pressure on *Podostemum ceratophyllum*?” we conducted an experiment in the Middle Oconee River at Ben Burton Park that entailed measuring

Podostemum stem length on uniquely identified rocks colonized with *Podostemum* and transplanted between high- and low-velocity habitats over multiple time periods. To explore the evidence that low-velocity conditions alone negatively impact *Podostemum*, we utilized cages to exclude consumers in low-velocity habitat, in both the Middle Oconee and Apalachee Rivers. To answer the question “Do large-bodied herbivores (e.g., geese, turtles, and crayfish) influence *Podostemum* biomass accrual?”, we conducted a multi-week consumer exclosure experiment using electrified consumer exclosures. Utilizing data from this experiment we then estimated the consumption rate of *Podostemum* by consumers.

Effects of Flow Velocity on Podostemum Stem Length

To assess the influence of water velocity on *Podostemum* stem length, we collected fifty medium-sized cobbles heavily colonized by *Podostemum*, hence-forth called substrates, on June 28th 2014. Discharge in the river was approximately 6 m^3s^{-1} according USGS gage 02217500, located 2.3 km downstream from the study site. Substrates were selected if *Podostemum* stem length was relatively uniform and longer than 3 cm. Each substrate was uniquely identified with colored zip-ties and randomly assigned to either a high-velocity or low-velocity treatment. We measured five randomly-selected stems of *Podostemum* from each substrate to estimate average beginning stem-length. Substrates were then distributed throughout the shoal, with 25 of the substrates placed in high-velocity locations (velocity greater than 0.5 $m\ s^{-1}$) and 25 placed in low-flow locations (velocity < 0.5 $m\ s^{-1}$). We measured water velocity and depth at each substrate location with a Marsh McBirney flowmate (model 2000) and wading rod. We retrieved the marked substrates and again measured five randomly-selected stems on each to assess the change in stem length after approximately 24 hours, 12 days and 56 days. On day 12, we randomly selected 10 substrates from the low-velocity treatment for transplant back into high-velocity locations. We also measured depth and velocity at substrate locations on days 12 and 56. River discharge was 4.5 and 3.5 m^3s^{-1} when substrates were recollected on days 12 and 56, respectively, varied from approximately 4.2 m^3s^{-1} to 31.1 m^3s^{-1} between days 1 and 12, and from approximately 1.1 to 48 m^3s^{-1} between days 12 and 56.

We tested for significant change in stem length in low- and high-water velocity locations after 24-hours and 12 days using a one-sample one-way T-test, with the *a priori* hypothesis that stem loss would be higher in low-velocity locations. We similarly used a one-way ANOVA, followed by a Tukey HSD test, to test for differences in change in stem length after 56 days among the three treatments: high-velocity, low-velocity and transplanted from low- back to high-velocity. Finally, we used linear regression to assess the correlation between changes in stem length and water velocity during the 12-day incubation.

Cage Experiments

We assessed the impact of short-term low velocity conditions on *Podostemum* by measuring change in stem length after 7 days using pairs of caged (to exclude larger bodied consumers) and un-caged substrates transplanted into low-velocity habitats in the

Middle Oconee and Apalachee rivers. Cages measured approximately 40 cm long with a diameter of 18 cm and were constructed of wire mesh having approximately 0.25 x 0.25 cm openings. Five randomly-selected stem lengths were measured on each substrate at the beginning and end of the experiment. Substrates were randomly assigned a paired treatment (caged or no-cage), and then placed within 1 m of each other in low-velocity locations (<0.5 m/s) at depths of 0.5 - 2 m. We used one-sample one-way T-test to assess change in stem length on caged and uncaged substrates. Substrates were originally collected from the Middle Oconee River at Ben Burton Park.

Electrified Consumer Exclosures

Five pairs of consumer exclosures were installed in the Middle Oconee River below the Tallassee Shoals Dam on July 9, 2015, so that each pair contained a treatment (electricity) and a control (no electricity). The Tallassee Shoals site was chosen because there was limited pubic access (making it safer to deploy the exclosures) and because *Podostemum* was present but mostly short (<2 cm tall), suggesting substantial grazing pressure. Exclosure locations were chosen based on the presence of relatively uniform coverage of *Podostemum*, depth, and flow velocity. Exclosures were made of 8 gauge copper wire, bent into an outer wire frame of 45 x 45 cm and an inner wire frame of 25 x 25 cm, similar to Pringle and Blake (1994). Exclosures were powered by one joule Speedrite 1000 Unigizer fence chargers connected with 12 gauge multi-strand copper wire and pulsed approximately every 1.5 seconds. Exclosures were held in place with epoxy and large stones to prevent the exclosures from being dislodged during high-discharge events. An electrified treatment was randomly assigned to one exclosure within each pair. Weekly, for 11 weeks, ten randomly-selected *Podostemum* stems were measured *in situ* (underwater) in treatment and control exclosures. On day 77 we turned off the power to the exclosures and collected *Podostemum* biomass. Mean water velocity and depth during the duration of the experiment in the control and exclosure treatments was 0.40 m s^{-1} (SE ± 0.03) and 16.3 cm (SE ± 1.6), and 0.36 m s^{-1} (SE ± 0.03), and 15.2 cm (SE ± 1.2), respectively.

A T-sampler, constructed from a cylindrical plastic container with the bottom removed, was used to collect all plant material from a 67.9 cm^2 area in the center of each exclosure by pressing the sampler firmly against the substrate and scraping all plant material loose. Plant material from each exclosure was placed into separate labeled bags and transported to the lab. In the lab, samples were vigorously washed in a 2mm sieve to isolate plant material from sediments, detritus, and invertebrates, and a 250µm sieve was placed below to catch dislodged materials. Plant material was then dried at 60ºC for 48 hours, weighed, ashed at 500ºC for four hours, and then weighed again to calculate the ash-free dry mass (AFDM) of each sample. Snails found in the sample were removed, counted, and stored in 70% ethanol.

A paired T-test was used to assess differences in *Podostemum* biomass between the treatment and control at 77-days, and to assess differences in snail biomass between treatments. We used the measurements of accumulated biomass on day 77 to estimate an average daily amount of biomass consumed as the difference between average final biomass (AFDM) in exclusion and control treatments divided by 77 days. We estimated biomass consumed per day by subtracting treatment biomass (AFDM) from control biomass (AFDM) then dividing by the duration of the experiment (77 days).

We used the weekly measurements of stem length in electrified and control exclosures to estimate stem growth rate and the percent of growth that was consumed. We assumed that growth was exponential, that observed change in stem length over time was the difference between growth and consumption (both as cm cm^{-1} d^{-1}), and that there was no loss to consumption in the electrified exclosures:

$$length_{i,\, j,\, t} = length_{i,o} * e^{r_{i,k}\ t} ,$$

where $length_{i,k,t}$ is the mean stem length measurement in exclosure *i* (1 or 2) in pair *k* (1 – 5), on day *t*. In this equation:

$$r_{i,k,} = growth\ rate_{i,k} - consumption\ rate_{i,k},$$

$$growth\ rate_{i,k} = mean\ growth\ rate + epsilon_{i}, \text{ and}$$

$$consumption\ rate_{i,k} = growth\ rate_{i,k} \text{ x mean proportion consumed}$$

where $epsilon_i$ is a random effect accounting for repeated measurements within exclosures through time. We fit this model to estimate mean growth rate and mean proportion of growth consumed using a Bayesian framework implemented with the Markov chain Monte Carlo software JAGS (Plummer, 2003), run using the R package “rjags” (Plummer, 2014; Team, 2014); code, priors and MCMC specifications are shown in Appendix B.

Results

Effects of Flow Velocity on P. ceratophyllum

Substrates transplanted to low-velocity habitats exhibited a 57% decrease in stem length after 24 hours compared to almost no change on substrates transplanted to high-velocity habitats. Beginning mean stem lengths were 7.6 and 8.0 cm in the high- and low-velocity habitats, respectively, and were reduced to 3.4 cm in the low-velocity locations after 24 hours (one-sample one-way T-Tests, Low flow: t(24) =-8.17, P > 0.001; High-flow: t(24)= 0.61, P = 0.72; Figures 4.2 a-d & 3a-b). Mean depth in the high and low flow treatments was 14.7 cm (SE ± 1.0) and 16.5 cm (SE ± 2.5.0), respectively.

After 12 days of *in situ* incubation, stem length was still significantly shorter in the low-velocity treatment compared with high-velocity (Figure 4.3b) and the overall change in stem length was significantly correlated with water velocity (y = 7.55x-6.64, adj. R^2 = 0.55, df = 43, P < 0.001; Figure 4.4). ANOVA results indicated significant recovery of stem length on day 56 by substrates in the reciprocal transplant (low- to high-velocity) treatment, such that *Podostemum* stems transplanted from low-velocity back to high-velocity locations were on average 2.8 cm longer than those that remained in the low-velocity treatment (Figure 4.5a), indicating an average growth rate of 0.05 cm day^{-1} in the transplanted treatment group. Total stem length was significantly different between the low-velocity locations and the *Podostemum* transplanted back into high-velocity locations (ANOVA *F*(2,127) = 64.93, P<0.001; Figure 4.5b) and there was as a significant difference in the change in stem length between the low- and transplanted-into-high- velocity locations, and the low- and high-velocity locations (ANOVA P < 0.001).

Cage Experiments

After 7 days in low water velocity habitat (mean = velocity 0.11 m s^{-1}) *Podostemum* stem length was severely reduced when the plant was accessible to consumers (Middle Oconee, t(4) = 4.86, P<0.01; Apalachee, t(4) = 21.05, P < 0.01; Figure 4.6a), but no significant change in stem length was observed when the plant was protected from consumers (Middle Oconee, t(4) = -0.24, P = 0.59 Apalachee, t(4)=0.78), P = 0.24; Figure 4.6b). In the non-caged treatments, stem length was reduced by 4.6 cm (SE ± 0.9) at the Middle Oconee site and by 8.6 cm (SE ± 0.4) at the Apalachee study site.

Electrified Consumer Exclosures

We found that consumer access significantly limited the accrual of *Podostemum* biomass. After 77-days, mean stem length was 4.1 times longer in the electrified than in the control (consumer access) treatments (4.3 and 1.1 cm respectively; Figure 4.7 b-c,) and mean AFDM was 1.9 times higher in the electrified exclosures compared with controls (170.5 ±36.9 and 87.6 ±15.8 g AFDM m^{-2} respectively; paired t-test, t (4)=3.2, P < 0.03; Figure 4.8). Only hydrobiid snails were present in the samples and mean snail abundance was significantly higher in the exclosure treatment compared with controls (means = 0.90 and 0.18 individuals cm^{-2}, respectively; P < 0.01; Figure 4.9).

Growth rate and Contributions to the food web

Differences in biomass accrual were apparent throughout the 77-d consumer exclusion vs. access experiment (Figure 4.10). We calculated an average growth rate of 0.026 cm cm^{-1} day^{-1} with 95% credible intervals between 0.013 – 0.039. Estimated mean proportion of *Podostemum* growth consumed was 85% (95% C.I. = 67-98%). Assuming this constant rate of consumption and converting stem length to biomass (using final biomass and stem measurements), we estimated 1.7 g DM m^{-2} day^{-1} (1.1 g AFDM m^{-2}) were contributed to the food web during the 77-d study.

Discussion

Our results supported our hypothesis that water velocity can mediate trophic interactions via increasing or decreasing herbivory pressure on *Podostemum*. Contrary to other studies that found increased water velocity decreased basal resource storage (Riis and Biggs, 2003; Francoeur and Biggs, 2006) we found that increased water velocity increased basal resource accrual. Additionally we found that when water velocity was reduced, there was a rapid release of stored basal resources, presumably into food webs. The magnitude of the influence that water velocity exerted on *Podostemum* was surprisingly large and we concluded that water velocity facilitates resource storage when velocities are relatively high and release when velocities are lowered. Collectively, these results support a conclusion that high water velocity habitats can mediate top-down trophic interactions and influence ecosystem structure and function by regulating benthic storage and resource flux into consumers.

Consumers exerted surprisingly strong effects on plant biomass by significantly reducing stem length in the low-velocity habitats and by consuming 85% of new growth as measured by the consumer exclosure experiment. Our results indicate *Podostemum* is a highly utilized resource in riverine habitats and that even short-term reductions in water velocity can reduce plant biomass via herbivory. These reductions in plant biomass may subsequently reduce habitat for invertebrates (Hutchens et al., 2004) and fishes (Connelly et al., 1999; Argentina et al., 2010), and alter contributions to detrital food webs (Nelson and Scott, 1962). Thus, the rapid loss of plant biomass during prolonged low-flow conditions may fundamentally alter lotic resource processing, and impacts may be propagated downstream in the form of reduced detrital export.

Regional climatic events such as long-term drought and high-discharge years may drive large fluctuations in stream resource storage and flux (Riis and Biggs, 2003; Suren and Riis, 2010). For example, during periods of high discharge and high benthic water-velocity, *Podostemum* biomass is predicted to increase substantially as a result of increased habitat (submerged stable substrates) for the plant within the channel as well as reduced herbivory pressure. This is in contrast to most other rooted macrophytes, which are expected to increase in biomass during periods of low flow as a result of decreased water velocity and scour (Suren and Riis, 2010; Wood and Freeeman, 2017). The increased habitat and reduced herbivory on *Podostemum* during periods of higher discharge would thereby increase resource storage in the benthos via the accumulation of plant biomass and could increase secondary production of invertebrates, fish and herbivorous vertebrates. Conversely, when discharge and velocity decrease, accumulated biomass is predicted to move rapidly into food webs though increased herbivory pressure and desiccation of exposed plant material. Reductions in *Podostemum* biomass and thus nutrient uptake in rivers experiencing low flow conditions may lead to increased nutrient spiraling length (Newbold et al., 1981). Reductions in *Podostemum* biomass and thus habitat for macroinvertebrates may also lead to reductions in secondary production (Grubaugh and Wallace, 1995; Hutchens et al., 2004) and resource flux into adjacent terrestrial habitats (Baxter et al., 2005). While prolonged periods of low flow may functionally extirpate the plant from river segments, recovery of plant biomass may be rapid if suitable habitat is restored and if roots (Pahl, 2009; Philbrick et al., 2015), seeds, (Philbrick and Novelo, 1994; Philbrick and Alejandro Novelo, 1997) or both, are present to facilitate recolonization.

The intense herbivory pressure observed in low velocity habitats indicates that the realized niche of *Podostemum* can be strongly controlled by consumers interacting with water velocity. This phenomenon was hypothesized by Wood and Freeeman (2017) and is evidenced by the persistence and growth of *Podostemum* in low-velocity habitats when protected from consumers. Supportive of this notion, the reciprocal transplant experiment showed that recovery of heavily grazed plants occurred relatively quickly once herbivory pressure was reduced by translocation into high-velocity habitats. This insight into the relationship between water velocity and herbivory may elucidate a key evolutionary strategy of Podostemaceae: utilization of habitats that restrict consumer access and herbivory, thus maximizing growth with minimal investment in chemical or physical defenses.

Lastly, while the classical view of riverine macrophytes has purported that herbivory pressure is minimal because of the low nutritional value of macrophyte, our results support the growing body of research that indicates that macrophytes are frequently consumed in substantial quantities (Lodge, 1991; Newman, 1991; Bakker et al., 2016) and contribute significantly to lotic carbon budgets and nutrient dynamics. While we did not specifically investigate elemental composition in this study, *Podostemum* is approximately 2.6% nitrogen and 36% carbon, with a mass g g^{-1} C:N ratio of approximately 13.5 (J.W. *unpublished*). Thus the mass C:N ratio of *Podostemum* is lower than most terrestrial plants (median = 25-30) and marine macrophytes (median = 24 – 28), and similar to other freshwater macrophytes (median = 12-16), although slightly higher than many submerged freshwater macrophytes (median = 8-12) (Bakker et al., 2016). A higher relative C content in *Podostemum* may be related to the physical stresses of growing in swift-water habitats where additional carbon rich structural molecules are necessary to minimize damage during high flow events. Nonetheless, the overall low C:N of *Podostemum* indicates that this macrophyte is a high-quality resource to aquatic and terrestrial consumers when they gain access to the plants. Primary consumers of *Podostemum* in our experiments likely include the herbivorous river cooter (*Pseudemys concinna*) (Buhlmann and Vaughan, 1991; Lagueux et al., 1995), crayfishes (*Cambarus* and *Procambarus* species), and Canada geese (*Branta canadensis*) (Parker et al., 2007), all of which are widely distributed in the eastern U.S. and observed in and around our study plots during the duration of the experiment. However, attempts to document unwater feeding were unsuccessful due to high water turbidity and frequent nocturnal or crepuscular feeding behavior. To the contrary, Canada geese were observed feeding on *Podostemum* upstream of one of our study sites on multiple occasions. Further investigation into the effects of specific consumers, and into the elemental composition of

P. ceratophyllum and other members of Podostemaceae would aid in modeling resource storage and flux in swift-water rivers. Furthermore, investigations into trophic relationships may elucidate previously unreported reciprocal subsidies between terrestrial and aquatic ecosystems.

Because *Podostemum* plays a unique role in eastern montane and piedmont rivers, these findings have implications for informing ecological flow management in the region (Poff et al., 2009). The oscillating pattern of resource sequestration (high flow) and mobilization (low flow) by *Podostemum* and its consumers is relevant to rivers that are managed in a prolonged or continuous low-flow state (e.g., by water extraction or diversion), or in a pulse-flow state (e.g., by hydropeaking power production), and could be utilized to better estimate ecosystem consequences resulting from anthropogenic climate change effects on inter-annual and seasonal flow dynamics. Low-flow conditions are expected to exhibit reduced *Podostemum* biomass but the effect of losing *Podostemum* on nutrient cycling is unclear. Managing for more natural flow regimes in flow-regulated rivers could restore *Podostemum* habitat in rivers where the plant has been extirpated (Wood and Freeeman, 2017), which in turn would increase habitat for macroinvertebrates and fishes, and may aid in managing for nutrient enrichment. Furthermore, because the members of the family Podostemaceae are found in swift-water habitats throughout South America, Africa, India and Asia, these finding have ecological implications for flow management, nutrient cycling, and climate predictions beyond eastern North American rivers.

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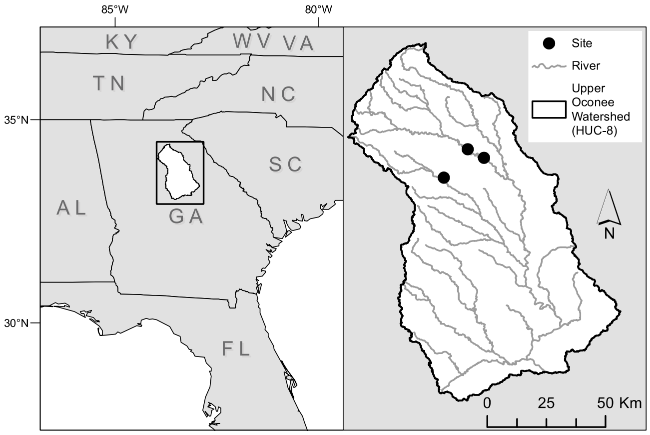

Figure 4.1. Map of the study locations. Experiments were conducted at two locations on the Middle Oconee and at one location Apalachee River near Athens Georgia.

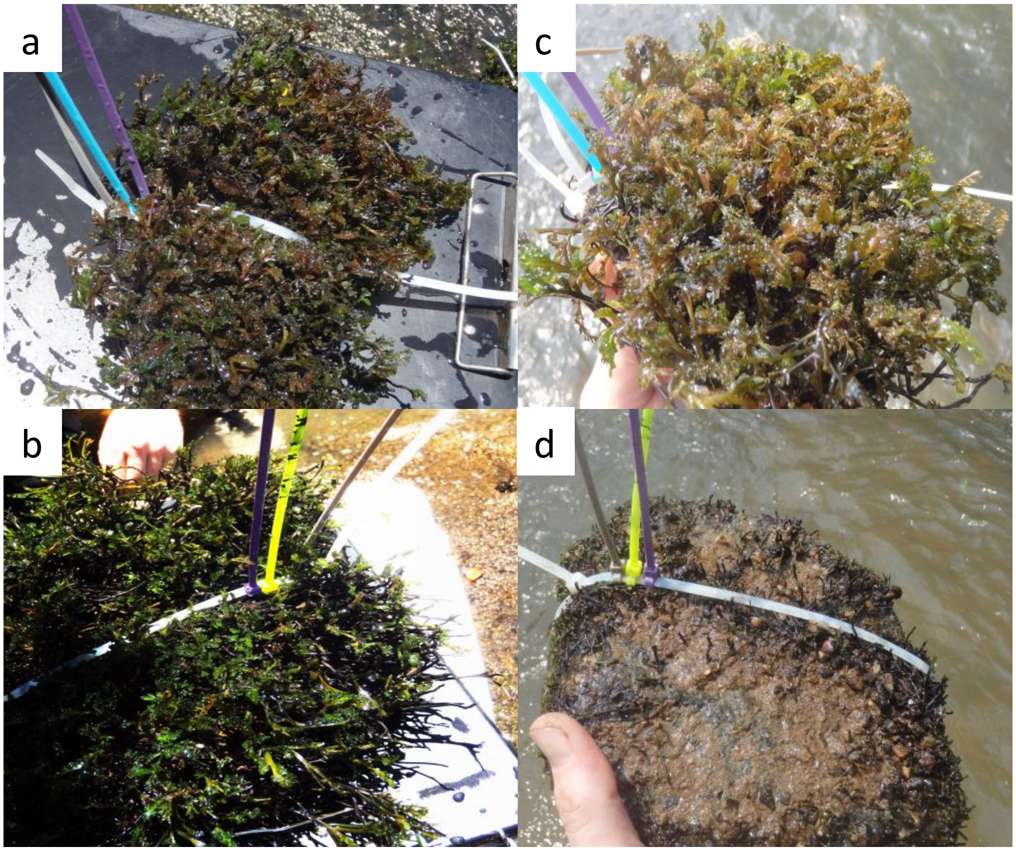

Figure 4.2. Rocks with *Podostemum ceratophyllum* removed from areas of the rivers with plentiful *P. ceratophyllum* before receiving a flow treatment (a, b) and after 24 hours in high flow treatment (c) and low flow treatment (d). Note the near complete loss of plant materials in panel (d).

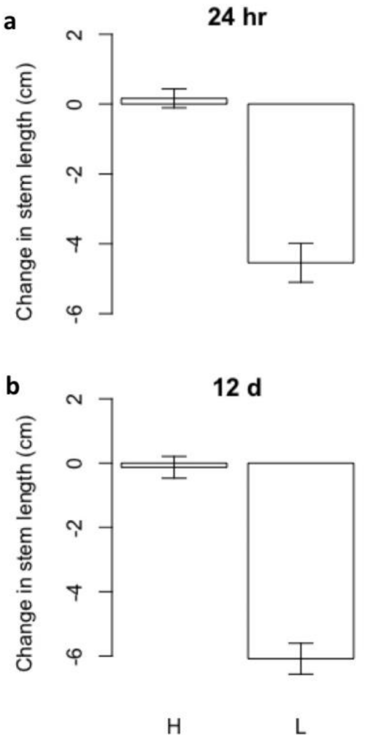

Figure 4.3. Change in *Podostemum* stem length after 24 hours (a) and 12 days () in high (H) or low (L) velocity treatment (p<0.001 in both; n=25 substrates per treatment). Error bars are +/- 1 SE.

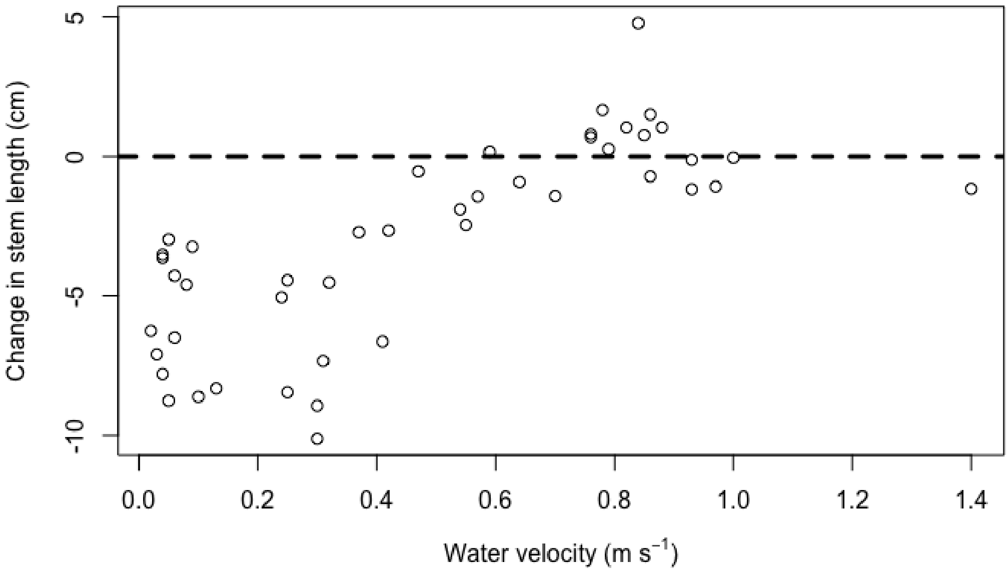

Figure 4.4. Change in stem length of *Podostemum ceratophyllum* 12 days after translocation in the Middle Oconee River, plotted in relation to water velocity measured on July 10, 2015.

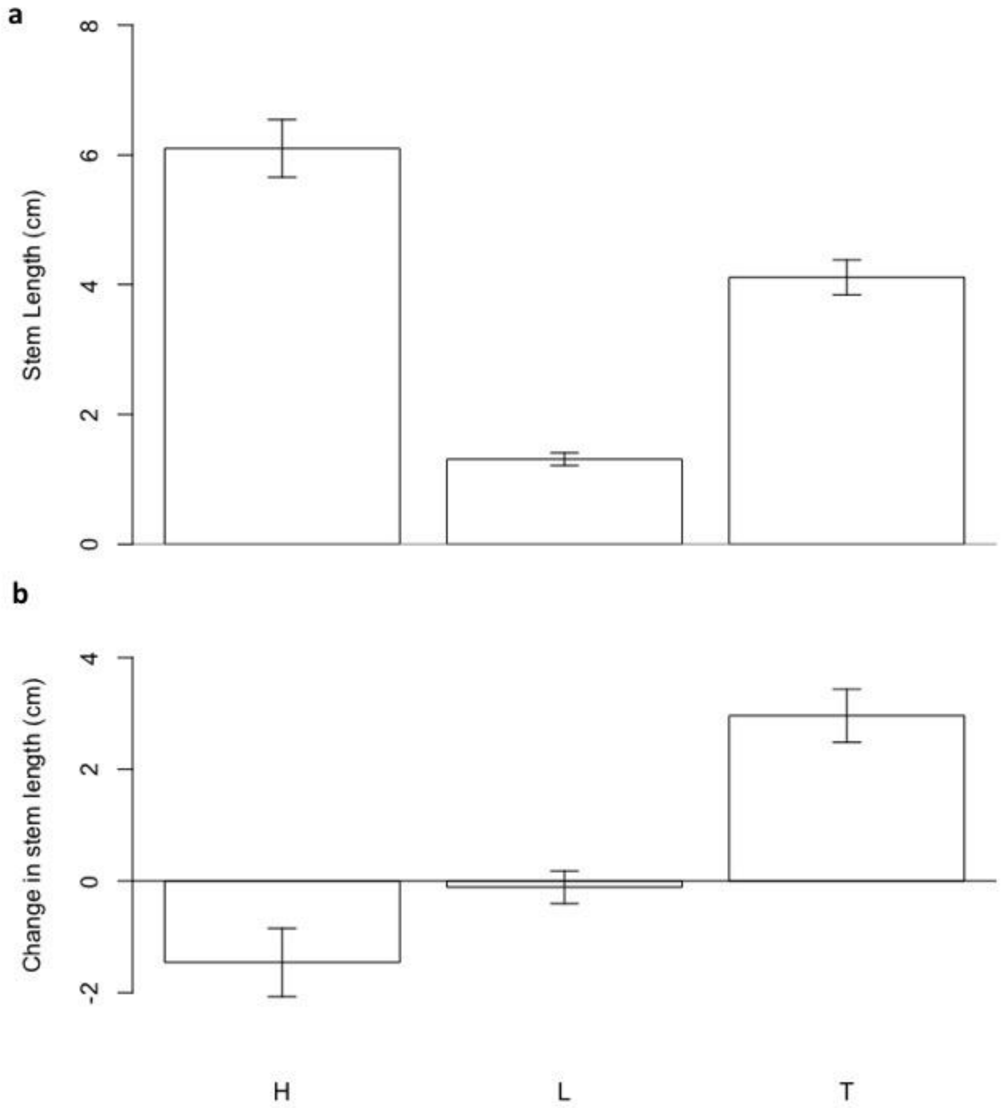

Figure 4.5. *Podostemum ceratophyllum* stem length at day 56 in a reciprocal transplant experiment (a), and change in stem length after 56 days (b) in the high (H), low (L) and transplant from low into high (T) water velocity treatments. The transplant group comprised samples that had been placed in a low flow treatment for 12 days and then moved to a high-flow treatment location. All treatments were significantly different in length from each other at 56 days (TukeyHSD, $P < 0.001$ all pairwise comparisons, and change in stem length was significantly different, between the low and transplant treatments ($P = 0.01$), and the high and low velocity treatments ($P < 0.001$). .

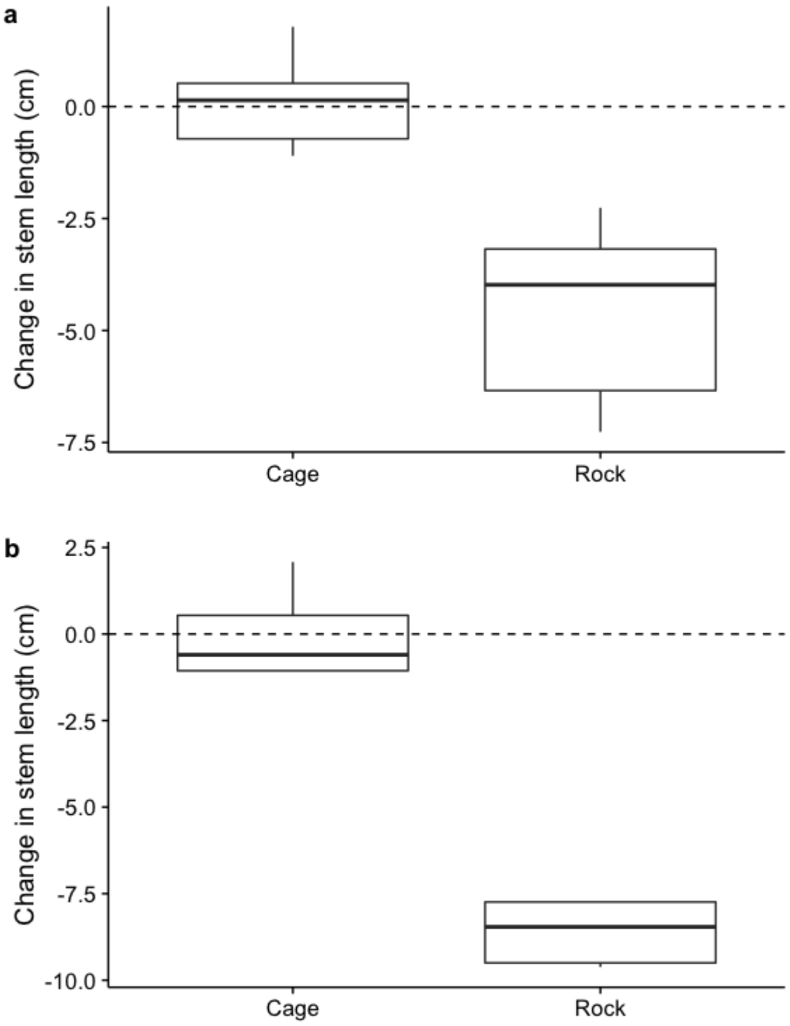

Figure 4.6. Results from the consumer cage exclosure experiment where consumers significantly reduced stem and stem length when *Podostemum ceratophyllum* was accessible. Change in stem length was not significantly different from zero in the consumer exclosure "Cage" treatments in the Middle Oconee River (a) and the Apalachee River (b) ($P > 0.05$ in both). In both study location stem length was significantly reduced in the control "Rock" treatment, which did not have exclosures ($P < 0.01$). Dashed line represents zero change in stem length.

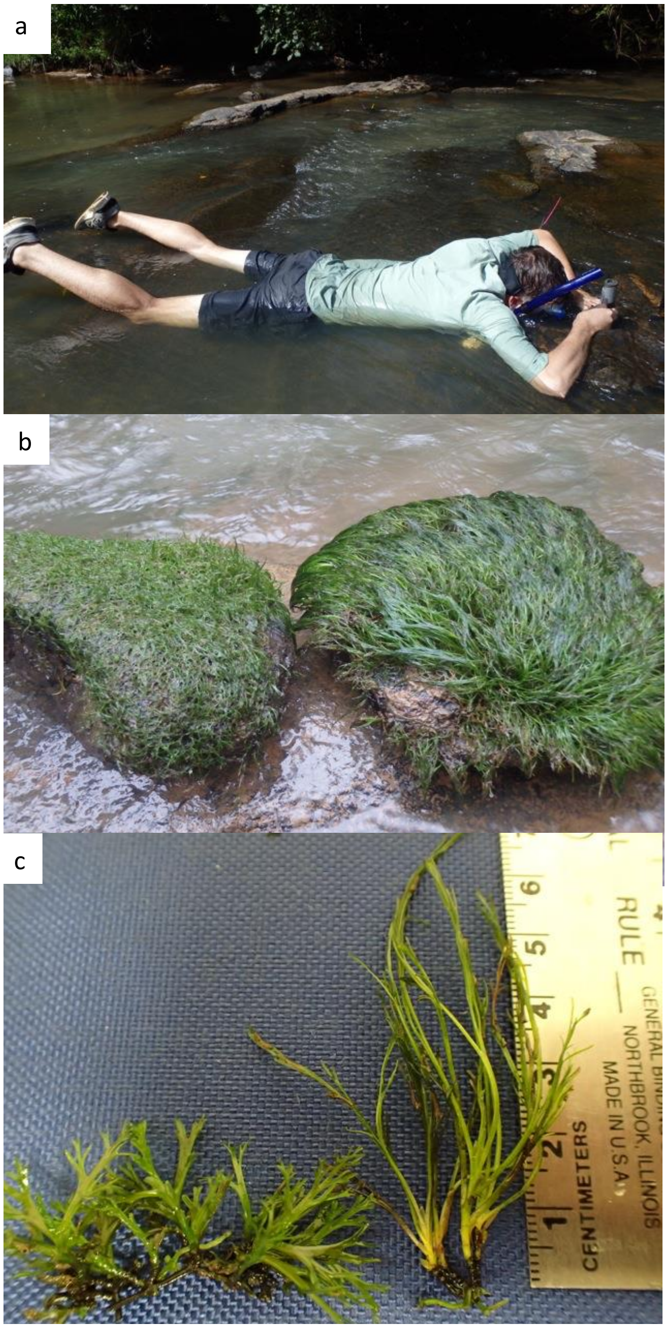

Figure 4.7. Measuring *Podostemum ceratophyllum* stem length on bedrock in consumer exclosure experiment in the Middle Oconee River (a), *P. ceratophyllum* from consumer exclosures (b-right) and control where consumers had access to *P. ceratophyllum* (b-left), and close up of *P. ceratophyllum* in control (c-left) and from the exclosure treatment (c-right) Significant differences in *Podostemum ceratophyllum* stem length and biomass were observed between control plots ($P < 0.05$). Note the differences between the leaf-length to leaf-width ratio. Small brown structures on leaves are chironomids cases. Photographs by James Wood (2015). Photograph of Jon Skaggs (a) measuring stem length underwater in consumer exclosures.

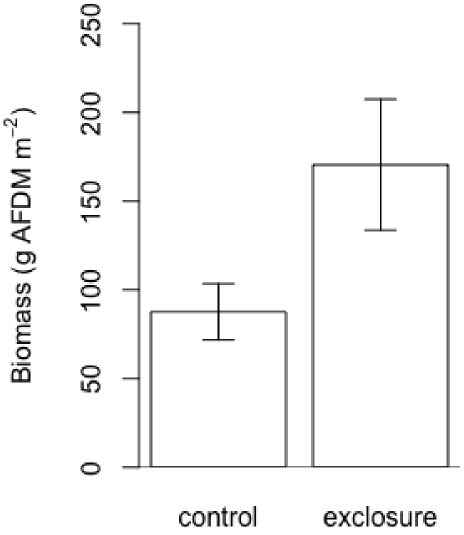

Figure 4.8. *Podostemum ceratophyllum* biomass (± 1 SE) after 77 days of a consumer exclosure compared with control plots where consumers had access to the plant (n = 5, *P* = 0.03).

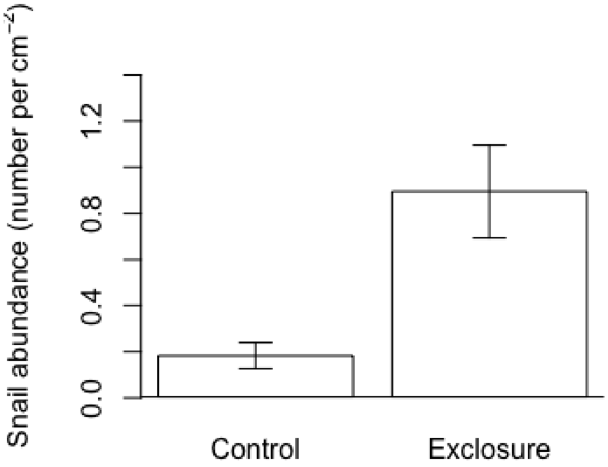

Figure 4.9. Snail abundance (number per cm^{-2}) was significantly higher in the consumer exclosure treatments than in the control treatments (t(4)=3.99, *P* =0.02).

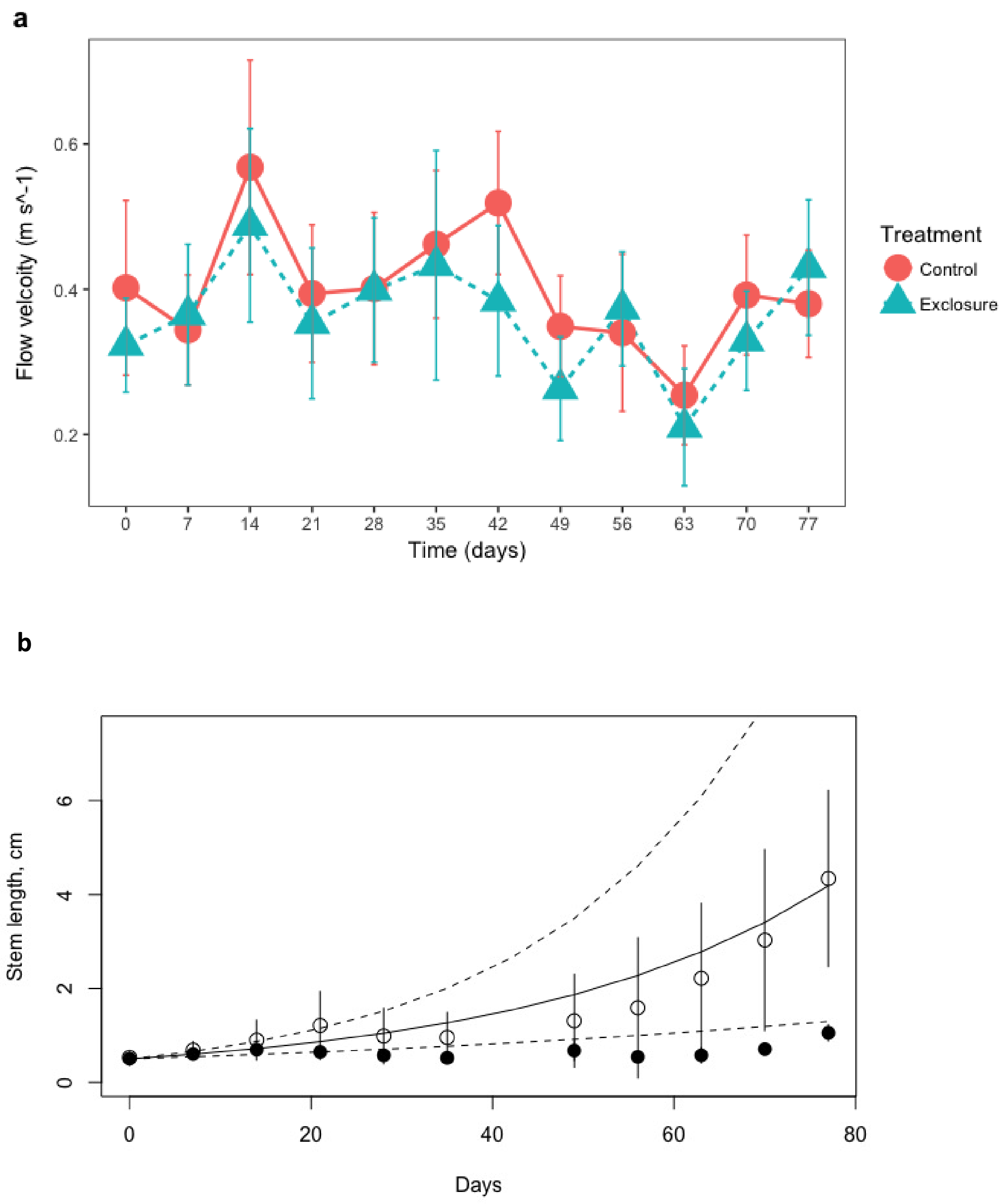

Figure 4.10. Water velocity (a) and mean *Podostemum* stem lengths in electrified (b), consumer exclusion (open circles) and non-electrified control (consumer access, filled circles) treatments during a 77-d experiment using 5 exclusion-access pairs. Error bars represent ± two standard errors. Line shows the mean predicted length (and 95% credible intervals, dashed) based on the average growth rate estimated from the consumer exclusion units.

CHAPTER 5

ELEMENTAL COMPOSITION OF THE WIDESPREAD RIVERINE PLANT (*PODOSTEMUM CERATOPHYLLUM* MICHX.) REFLECTS LAND USES ACROSS EASTERN NORTH AMERICA: IMPLICATIONS FOR ASSESSING ANTHROPOGENIC NITROGEN AND TRACE METAL[1]

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[1] Wood, J. L., M. C. Freeman, D. Leasure, T. Maddox, K. Lofit, S. J. Wenger, A. D. Rosemond, and J. W. Skaggs. 2017. To be submitted to Science of the Total Environment

Abstract

Ecological stoichiometry is helping to elucidate the effect of anthropogenic nutrient enrichment on ecosystems worldwide. In freshwater systems, watershed land use influences surface water quality and may alleviate stoichiometric constraints on primary production and biogeochemical cycling, but the effects of land use on benthic resource quality remain unclear. We used the widespread riverine macrophyte, *Podostemum ceratophyllum*, to test for effects of land use on the plant's elemental composition, including carbon (C), nitrogen (N), phosphorus (P), δ^{15}N and the trace metals cadmium (Cd), sodium (Na), and zinc (Zn). *Podostemum ceratophyllum* is native throughout the montane and piedmont regions of eastern North America, where it grows submerged and attached to stable benthic substrates. The plant is also consumed by a variety of vertebrate and invertebrate herbivores. We found that reductions in forest cover were correlated with increased δ^{15}N in *Podostemum* tissue. Urbanization and watershed development were correlated with %P of plant tissue and with molar ratios of C:N, C:P. and N:P, although N content was not strongly correlated with land use. Cadmium concentration in plant tissue increased with impervious surface cover and high intensity development, and Zn concentration increased with medium and high intensity development. Latitude and high intensity development were positive correlated with Na concentration. These results support the conclusion that land use is reflected in the elemental composition of *P. ceratophyllum,* that land use can influence benthic resource quality and the loading of trace metals in food webs, and that *Podostemum* can be used as an indicator of anthropogenic nitrogen loading in rivers.

Introduction

Ecological stoichiometry is increasingly utilized as a tool to better understand ecological interactions and the flow of materials through the environment, and to assess the influences of anthropogenic nutrient enrichment on biogeochemical cycles (Elser et al., 2010, Rosemond et al., 2015). Ratios of carbon (C), nitrogen (N) and phosphorus (P) are especially important because of their role in organismal growth and their influence on food webs (Hessen, 1997; Elser et al., 2000), metabolism (Elser et al., 2010) and decomposition (Manning et al., 2016, Kominoski et al., 2015). The availability of nitrogen and phosphorus limits autotrophic production in most ecosystems. However, nutrient enrichment of freshwater systems is occurring globally, via agricultural fertilizers and manure inputs, municipal sewage influent, and atmospheric deposition. Unfortunately, nutrient enrichment of freshwater systems often has negative implications for human health and ecosystem services (Harpole et al., 2011; Paerl et al., 2011).

Stable isotope analysis of N is widely used to explore ecological interactions because $\delta^{15}N$ enrichment predictably increases with each link of the food chain. Enrichment of $\delta^{15}N$ in primary producers has also been shown to be caused by municipal sewage and leaking sewer lines (Kaushal et al., 2006), and by runoff from agriculture fields and livestock production (Anderson and Cabana, 2005). As a result, biofilms (Peipoch et al., 2012), macrophytes, and higher level consumers (Anderson and Cabana, 2007) have been used to assess the degree of anthropogenic N enrichment in aquatic systems. However, differences in regional species pools and variations in consumer trophic position (Kristensen et al., 2016) can obscure the effects of N enrichment on producers and consumers. Therefore utilizing a single autotrophic species to assess nutrient enrichment and anthropogenic impacts over large geographic areas has value for watershed mangers and ecological modeling.

The widespread aquatic plant family Podostemaceae occurs in rivers in the Americas, Africa, and Asia (Tippery et al., 2011; Koi et al., 2015). The ecology of these plants has received little attention, in part due to challenges associated with morphological identification and difficulties accessing their swift-water habitats (Wood and Freeeman, 2017). *Podostemum ceratophyllum* Michx. is the only North American member of the family north of Mexico, and the plant grows submerged and attached to stable substrates in the eastern piedmont and montane regions (Philbrick and Crow, 1983). The plant specializes in habitats ranging from sunlit waterfalls in headwater streams to open-canopy rivers (Philbrick and Novelo, 2004) where the plant can cover a substantial proportion of the benthos. Wood and Freeeman (2017) proposed that *Podostemum ceratophyllum*, henceforth called *Podostemum*, is a foundation species in eastern U.S. rivers because of its large geographic range, and the plant's substantial positive influence on secondary production (Grubaugh and Wallace, 1995; Hutchens et al., 2004), fish species occurrence (Connelly et al., 1999; Argentina et al., 2010b) and substrate stability (Argentina et al., 2010a; Duncan et al., 2011). Additionally, *Podostemum* forms an important component of lotic food webs by contributing substantially to the detrital pool through seasonally senesced tissue (Nelson and Scott, 1962; Hill and Webster, 1984), and through direct consumption by a variety of vertebrate and invertebrate species (Parker et al., 2007; Wood and Freeeman, 2017). However, elemental composition of *Podostemum* (Adams et al., 1973; Heisey and Damman, 1982) and the effect of land use on tissue composition has only tangentially been investigated and the plant has yet to be assessed for its usefulness as an indicator of nutrient enrichment.

In this study we assessed correlations between human land uses and the elemental composition of *Podostemum ceratophyllum*. Our goals were (*i*) to determine if $\delta^{15}N$ concentration in plant tissue is reflective of land use across eastern North America, and (*ii*) to assess if changes in molar C:N:P ratio and Cd, Na and Zn concentrations are correlated with land use.

Methods

Between 2014 and 2016, we collected *Podostemum* from 34 rivers in 11 eastern U.S. states: CT, GA, MA, ME, NC, NH, SC, TN, VA, VT, and WV (Figure 1). Sampling locations included sites from the U.S. Environmental Protection Agencies' National Aquatic Resources Survey (NARS) and additional opportunistically sampled locations. We reviewed data on benthic macrophytes, stream order, and water velocity in the NARS database and then chose sites based on factors including accessibility, previously observed populations of *Podostemum*, and physical characters that correlate with *Podostemum* occurrence and that were observable through satellite imagery (i.e., river width sufficient to allow light to reach the benthos, geographic location within montane or piedmont regions, and riffle/shoal/cascade habitat sufficient to provide stable substrate). We opportunistically collected additional samples of *Podostemum* from rivers in CT, GA, MA, NC, WV to increase sample size and to provide greater within-river system replication.

Sample collection occurred as follows. Substrates colonized by *Podostemum* were located in the channel, then collected and vigorously agitated underwater to remove sediments and foreign material from the plant. *Podostemum* stems were cut just above the root, and placed into a clean sample container with a secure lid. Samples were collected from at least three locations within the channel whenever possible and combined to create a single composite sample. Containers were then placed on ice, transported to the lab and frozen at -80ºC. Samples were processed for analysis by washing with deionized water over a 2 mm sieve, and then inspecting plant material at 8x magnification to remove any remaining debris, algae, or macroinvertebrates. Cleaned material was then freeze-dried and ground before being analyzed.

δ^{15}N, C:N and metals analysis

Approximately 1.5 mg of dried plant material was used for δ^{15}N, %C and %N analysis. Molar C:N was calculated as C:N = (%C / %N)* (atomic weight of N/ atomic weight of C) while mass ratio was calculated as %C / %N. For metals analysis approximately 0.1g of dried plant tissue was acidified with 2.25 ml concentrated nitric acid and 0.5 ml concentrated hydrogen peroxide and heated for 16 hrs. Samples were then diluted and analyzed for metal concentrations using inductively coupled plasma mass spectrometry (ICP-MS) and flame mass spectrometry (MS).

Land Cover and Geographic Information

GIS-based predictor variables were created using an automated process called GeoData Crawler (Leasure, 2014) driven by Python programming and the Esri ArcGIS toolbox (Python, 2012; Esri, 2014). Streams were delineated based on the NHD+ flow accumulation raster (USGS, 2012; USEPA, 2013), defining streams as raster cells with greater than 3 km^2 drainages. Sites were snapped to streams before delineating their watersheds.

Land cover at each site was assessed based on the 2011 National Land Cover Database (NLCD), (Jin et al., 2013). Land use was calculated as a percent of the watershed in (NLCD classification identity numbers): Forest = deciduous (41) + evergreen (42) + mixed (43); OD = open development (21), <20% impervious surface; LID = low intensity development (22), 20-49% impervious surface; MID = medium intensity development (23), 50-79% impervious surface; HID = high intensity development (24), 80-100% impervious surface; Developed (Dev) = LID+ MID + HID; Agriculture (Ag) = Pasture/hay (81) + Cultivated crop (82); and non-Forest = Ag + Dev + OD (Appendix C). Using the 2011 NLCD, we also calculated average watershed impervious surface cover (ISC).

Statistical Methods

We constructed linear mixed-effects models using the R package LME4 to investigate correlations between *Podostemum* elemental composition and land use metrics, plus a null model. We separately modeled 10 response variables (δ^{15}N, C, N, P, Cd, Na, Zn, and molar ratios of C:N, C:P, and N:P) in relation to each of nine land use metrics (modeled individually to isolate effects on *Podostemum* composition) that were arcsine transformed, scaled and centered. For Na, we took our top land use model and added latitude (scaled) as a predictor in the model because of an *a priori* hypothesis that Na would have a north-south influence because of increased road salt use. All models included river identity as a random effect on the intercept. AICc scores (Burnham and Anderson, 2004), marginal R^2 (variation explained by fixed effects only) and conditional R^2 (as a measure including river identity as a predictor, i.e., fixed + random effects) values were calculated to assess model fit between land uses and response variables (Nakagawa and Schielzeth, 2013). Unless otherwise noted, R^2 refers to marginal R^2 values.

Results

Carbon, nitrogen, phosphorus and δ^{15}N

Across all sites, %C in *Podostemum* tissue averaged 36.4% (SE ± 0.51) while mean %N was 2.7% (SE ± 0.07), and %P averaged 0.17% (SE ± 0.01; Table 1). Open Development (OD) exerted a negative effect on %C (Figure 2b) and was the top AICc model, with 42% of the model weight; OD explained 14% of the variation (R^2) in the data. Increasing Ag increased %C, although the correlation with Ag was poor ($R^2 = 0.07$). However, there was relatively strong grouping of the data by river system (Ag conditional $R^2 = 0.41$). Percent N was negatively correlated with Forest, and forested land cover was the top AICc model for %N, holding 35% of model weight. Forest was the only land use with confidence intervals that did not cross zero in models for %N (Table 2, Figure 2c). High intensity development (HID) was the top AICc model for %P, holding 51% of the model weight, and HID was relatively strongly correlated with increasing P content ($R^2 = 0.24$) (Figure 2d). Other categories of development, MID ($R^2 = 0.21$) and ISC ($R^2 = 0.20$) were also correlated with increasing %P of plant tissues. Table 3 includes land use means, standard deviation (SD), and range, and calculations to relate 1 SD of transformed land use data to untransformed land use effect on *Podostemum*.

$\delta^{15}N$ varied widely, ranging from 0.85 to 14.0, with mean of 7.06 (SE ± 0.35; Table 1). AICc rankings placed forested land cover as the best predictor of $\delta^{15}N$, holding 96% of the model weight (Table 2). Increasing forest cover was correlated with reductions in $\delta^{15}N$, while increasing development and ISC were correlated with increasing $\delta^{15}N$ (Figure 2a), with a potentially large influence from HID (mean $\delta^{15}N$ effect from HID = 9.41). R^2 values indicated that forest cover explained 22% of the variation observed in $\delta^{15}N$.

Molar Ratios

Mean molar C:N was 16.2:1 (SE ± 0.3), C:P was 642.7:1 (SE ± 30) and N:P was 39.5:1 (SE ± 1.6; Table 1). AICc rankings indicated percent OD was the best predictor of molar C:N ratio, accounting for the 42% of the weight across all models, while Ag was the second best model with 21% of model weight. Models indicated that increasing OD was associated with a decrease in the C:N ratio ($R^2 = 0.14$; Table 2, Figure 3a), while Ag was associated with an increase in the C:N ratio, although the 95% confidence interval included zero (mean effect = 0.86, confidence interval = -0.02 – 1.77, $R^2 = 0.07$).

Molar C:P was negatively correlated with HID ($R^2 = 0.19$) and HID held 31% of AICc model weight, followed by the combined development category (Dev) with 16% ($R^2= 0.17$) and OD with 14% of model weight ($R^2=0.17$). Development negatively influenced C:P while increasing forest cover was positively correlated with increasing C:P ($R^2 = 0.15$; Table 2, Figure 3b).

Molar N:P was negatively correlated with HID ($R^2 = 0.25$) and HID was the best-supported AICc model predicting N:P followed by MID ($R^2 = 0.22$) and ISC ($R^2 = 0.24$), with 37, 21 and 20% of the model weight respectively (Table 2, Figure 3c). Forest, however, was positively correlated with N:P ratio ($R^2 = 0.07$) and the 95% confidence interval did not include zero (0.43 – 7.60), indicating that as forest cover increased the amount of N in plant tissue increased relative to the amount of P in tissue.

Trace metals

Cadmium (Cd) averaged 1.2 $\mu g\ g^{-1}$ (SE ± 0.2) while mean Na and Zn concentrations were 2898.90 $\mu g\ g^{-1}$ (SE ± 225.15) and 137.1 $\mu g\ g^{-1}$ (SE ± 9.0), respectively (Table 4). Impervious surface cover and urban development in the watershed (ISC, Dev, MID, HID) were correlated with increasing Cd concentration in plant tissue (Table 2; Figure 4a). Impervious surface cover explained 15% of the variation in Cd and was the predictor in the top AICc model, with 47% of the model weight, followed by MID ($R^2 = 0.14$) with 39% of the model weight across all models.

Sodium (Na) was marginally correlated with development metrics, and was most strongly correlated with ISC, although the 95% confidence interval included zero (Table 2, Figure 4b). Although ISC was the top AICc model it only explained 5% ($R^2 = 0.05$) of the variation observed in the data. However, when we included latitude in the model with ISC, predictive strength increased substantially ($R^2 = 0.19$). Confidence intervals for latitude did not cross zero (Table 2) and indicate that *Podostemum* collected from more northern regions had increased concentrations of Na in the tissue.

Forest was the top AICc model for Zn, followed by MID and both were correlated with an increase in Zn concentration ($R^2 = 0.06$ for both; Table 2, Figure 4c). Forest held 53% of the model weight, while MID held only 14% of the weight across all models. However, the estimated mean effect was larger in MID compared with Forest, 56.34 and 22.95, respectively, but the 95% confidence interval for Forest was much smaller, 6.58 – 39.20, vs., 5.35 – 107.17 for MID.

Discussion

Macrophytes are increasingly being recognized for their influence on nutrient cycling and for their role in food webs in freshwater ecosystems. *Podostemum* specifically occupies a unique role among macrophytes influencing the structure and function of freshwaters because the plant sequesters nutrients solely from the water column as it attaches to rocks instead of rooting into benthic sediments. Our results support the hypothesis that land use affects the elemental composition of *Podostemum*, and provide evidence that land use influences basal resource quality and potentially toxicity in eastern rivers. Because *Podostemum* is a foundation species of eastern rivers (Wood and Freeeman, 2017) and is consumed by a variety of vertebrate and invertebrate herbivores (Parker et al., 2007), changes in resource quality and toxicity could impact higher trophic levels and have effects that propagate though freshwater ecosystems and into adjacent terrestrial systems (Baxter et al., 2005).

Our results indicate that *Podostemum* δ^{15}N is significantly influenced by reductions in forest cover, and supports our hypothesis that *Podostemum* can be utilized as an indicator of human-derived nitrogen pollution in rivers. We found that for every 1.5% percent increase in HID, we saw an increased in δ^{15}N of 9.41 ‰ (Table 3, Figure 2). While δ^{15}N has been used to track anthropogenic inputs of nitrogen in biofilm communities (Kaushal et al., 2006) and in riparian plants (Kohzu et al., 2008), we believe this is the first study to examine δ^{15}N in rivers using a single plant species on a multi-region scale. Using *Podostemum* as an indicator of anthropogenic N pollution may provide new insight into anthropogenic influence on nutrient cycling because *Podostemum* is a long-lived species and is resistant to scour compared with biofilms, thus integrating water quality conditions over a longer period of time. Additionally, because collection of *Podostemum* requires no specialized equipment, the plant may be useful in citizen-science water quality monitoring programs.

Collectively these data indicate that with respect to C and N, *Podostemum* is similarly nutritious compared with other freshwater macrophytes and algae, and that land use is correlated with shifts in the carbon to nitrogen ratio. Open development was negatively correlated with %C and C:N ratio, while increasing Ag in the watershed correlated positively with %C. The specific mechanism behind the land use effect on %C is unclear but may relate to increased scour, or to herbivory and light availability. Percent C of *Podostemum* was similar (< 10% difference) to values reported by Xia et al. (2014) from macrophytes in eastern China, and by Fernández-Aláez et al. (1999) from macrophytes in shallow south Europe lakes.

Nitrogen (%N) of *Podostemum* was similar (within about 0.02%) to concentrations observed in submerged macrophytes by Xia et al. (2014) but higher than values (<1% to ~2.4%) reported by Fernández-Aláez et al. (1999) and Yan et al. (2016; 2.0%., freshwater macrophytes). Compared with freshwater periphyton, *Podostemum* %C and %N was substantially higher (periphyton %C = ~8%, %N = ~0.6%), however, C:N ratio was similar (periphyton mean molar = ~15) (Stelzer and Lamberti, 2002). Furthermore, *Podostemum* mean mass C:N ratio of 13.6 g g^{-1} is slightly higher than the median C:N ratio for submerged freshwater macrophytes of 8-12 g g^{-1} reported by Bakker et al. (2016).

High intensity development within the watershed increased *Podostemum* P concentration and decreased C:P, indicating increased storage of P in plant tissue. *Podostemum* C:P was nearly twice that reported by Xia et al. (2014) for submerged macrophytes, likely because, as authors note, there were high concentrations of P in the local environment of the Xia et al. study due to untreated sewage and over- application of fertilizers on agricultural land. Conversely, we found a high N:P ratio (mass = 15.8, molar = 39.5) in *Podostemum*, higher than the N:P mass ratio of 7-13 reported by Yan et al. (2016) and Xia et al. (2014). Collectively, the correlations we found between land use and *Podostemum* %C, %P, and C:P, but not %N, and the relationship between

Podostemum %N and %P support the conclusion by Yan et al. (2016) that P accumulates faster than N in freshwater ecosystems under anthropogenic influence.

The maximum concentrations of metals found in *Podostemum* tissues raise questions, but not clear answers, about the plant's bioaccumulation of trace metals. Impervious surface was correlated with an increase in Cd concentration, and measured Cd concentration in *Podostemum* was as high as 10.6 µg g^{-1}. Effects of diet-born Cd have been observed at concentrations as low as 0.043µg g^{-1} in the algivorous mayfly *Centroptilum triangulifer* (Xie et al., 2010), however, effects on consumers have more frequently been observed at concentrations closer to 0.5 µg g^{-1} (DeForest and Meyer, 2015). In vertebrates, Cd toxicity may be due to disruption of copper and zinc metabolism (Hatakeyama and Yasuno, 1982; Weber et al., 1992) but mechanisms behind toxicity are unclear. In this study the median Cd burden of *Podostemum* was 0.8 µg g^{-1}, raising concerns that consumers feeding on *Podostemum* have the potential to be exposed to toxic levels of Cd, but we are unaware of Cd toxicity studies directed towards our suspected primary consumers of *Podostemum* (i.e., aquatic turtles, crayfishes, grazing birds and mammals).

Sodium concentrations in freshwater have been correlated with chloride (Daley et al., 2009), and salinity concentrations in eastern, especially northeastern, freshwater systems are increasingly reaching toxic levels (Kaushal et al., 2005; Coles et al., 2012). Sodium can be washed into rivers from the use of road salts and deicers, or through municipal sewage inputs, but sources of Na may vary within and among regions. The correlation between increasing Na in *Podostemum* and latitude is supportive of our concurrent findings that anthropogenic activities influence the elemental composition of

Podostemum. While we did not measure Cl^- in plant tissue, high chloride concentrations in river water may be a factor in the apparent decline of *Podostemum* throughout much its range (Wood and Freeeman, 2017).

We found that MID and Forest were correlated with increased Zn loading in *Podostemum*. We speculate that this duality may be due, in part, to slower growth rates in highly forested watersheds with lower nutrient availability (resulting in higher metal concentration in plant tissue), whereas in urbanized watersheds with higher surface water availability of nutrients and metals, bioaccumulation of Zn is mitigated by higher growth rates. High concentrations of diet-born Zn have been shown to exert reproductive harm on invertebrates (Hook and Fisher, 2002) at concentrations as low as ~3 $\mu g\ g^{-1}$, but effects are more frequently observed at concentrations closer to 20 $\mu g\ g^{-1}$ (DeForest and Meyer, 2015), an order of magnitude lower than the mean concentrations observed this study *Podostemum*. *Podostemum* thus appears to readily accumulate Zn at levels high enough to negatively affect consumers. However, the effects of metal toxicity on organisms can vary widely and are influenced by the identity of the organism, exposure route, molecular state, and binding complex (DeForest and Meyer, 2015), thus measurements of concentrations in plant tissue may not adequately reflect toxicity. Nonetheless, although herbivory on *Podostemum* is still poorly understood, increased metal loading in rivers may elevate concentrations of Cd, Zn and other trace metals in plant tissue to harmful concentrations for consumers feeding on this widespread macrophyte.

We have presented evidence that land use is reflected in the elemental composition of *Podostemum,* and specifically that elements that are commonly associated with urbanization (Cd, N, Na, P, Zn) can accumulate in *Podostemum*. Additionally we have shown that increases in impervious surfaces and associated watershed development, and the loss of forest cover, are correlated with changes in basal resource quality, which likely contribute to increased trace metal loading in benthic food webs. Because the plant family Podostemaceae is a globally distributed family, growing in rivers across North, Central and South America, Africa, and Asia, utilization of these plants as indicators of land use and changes in benthic resources may be a viable research and management tool. Because the rate of urbanization is increasing in many areas of eastern North America and around the world, additional means to quantify the effects of land use on basal resource quality and toxicity may be useful to managers. Lastly, while the major drivers of *Podostemum* decline in North American are currently unclear, the loss the plants from river systems has the potential to fundamentally alter nutrient cycling and benthic food webs in the rivers where the plant occurs. Thus, the restoration of extirpated populations may increase ecosystem services provide by rivers by sequestering water column nutrients and metals. However, if high metals loads are present in surface water, *Podostemum* may facilitate their transfer into the food web at concentrations harmful to consumers.

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Table 5.1. $\delta^{15}N$, Percent carbon (C), nitrogen (N), phosphorus (P) and stoichiometric molar ratios of *Podostemum ceratophyllum* collected between 2013 and 2016. Molar ratios are the averaged ratio from all samples. To convert to a mass ratio, use the equation mass ratio = (%) / (%).

	$\delta^{15}N$	%C	%N	%P	C:N	C:P	N:P
Mean	7.06	36.42	2.68	0.17	16.20	642.7	39.52
Min	0.85	27.65	1.90	0.05	12.32	197.0	14.18
Median	7.08	37.19	2.65	0.17	15.88	603.4	40.05
Max	14.04	43.14	3.91	0.45	21.81	1184.0	73.55

Table 5.2. Table of land use and model AICc ranking, lower confidence intervals (LCI) and upper confidence intervals (UCI), marginal and conditional R^2 values, and mean effect estimate. Predictor variable were asin transformed and scaled. Land use codes are as follows, Ag = Agriculture, OD = Open Development, LID = Low Intensity Development, Forest = Forest Cover, ISC = impervious surface cover, MID = Medium Intensity Development, HID = High Intensity Development, Dev = LID+MID+HID, not_Forest = Ag + Dev.

	land cover	**Model**	**K**	**AICc**	**Delta_AICc**	**AICcWt**	**Cum.Wt**	**LL**	**LCI**	**UCI**	**Marg. R^2**	**Con. R^2**	**Mean**
$\delta^{15}N$	**Forest**	**4**	**4**	**269.31**	**0.00**	**0.96**	**0.96**	**-130.29**	**-2.39**	**-0.97**	**0.22**	**0.70**	**-1.70**
	LID	**2**	**4**	**278.93**	**9.62**	**0.01**	**0.97**	**-135.10**	**0.81**	**4.35**	**0.14**	**0.44**	**2.55**
	Dev	**10**	**4**	**279.25**	**9.94**	**0.01**	**0.98**	**-135.26**	**1.05**	**5.98**	**0.13**	**0.44**	**3.47**
	HID	**5**	**4**	**279.55**	**10.24**	**0.01**	**0.98**	**-135.41**	**2.72**	**16.29**	**0.13**	**0.44**	**9.41**
	OD	**9**	**4**	**279.59**	**10.28**	**0.01**	**0.99**	**-135.42**	**0.42**	**2.52**	**0.16**	**0.38**	**1.45**
	ISC	**7**	**4**	**280.08**	**10.77**	**0.00**	**0.99**	**-135.67**	**0.91**	**6.31**	**0.13**	**0.47**	**3.38**
	not_Forest	**8**	**4**	**280.35**	**11.03**	**0.00**	**1.00**	**-135.80**	**0.44**	**3.29**	**0.13**	**0.48**	**1.82**
	MID	**3**	**4**	**280.60**	**11.28**	**0.00**	**1.00**	**-135.93**	**0.72**	**5.70**	**0.11**	**0.44**	**3.16**
	null	1	3	284.67	15.36	0.00	1.00	-139.12	5.63	7.58	0.00	0.30	6.66
	Ag	6	4	286.45	17.14	0.00	1.00	-138.86	-0.41	0.94	0.01	0.34	0.24
C	**OD**	**9**	**4**	**321.89**	**0.00**	**0.42**	**0.42**	**-156.57**	**-3.35**	**-0.27**	**0.14**	**0.28**	**-1.90**
	Ag	6	4	323.30	1.41	0.21	0.63	-157.28	-0.02	1.77	0.07	0.41	0.86
	null	1	3	324.67	2.78	0.10	0.73	-159.12	34.66	37.44	0.00	0.38	36.07
	forest	4	4	326.09	4.20	0.05	0.79	-158.67	-1.78	0.67	0.02	0.46	-0.59
	ISC	7	4	326.54	4.66	0.04	0.83	-158.90	-2.53	5.01	0.01	0.38	1.24
	not_Forest	8	4	326.67	4.78	0.04	0.87	-158.96	-1.43	2.58	0.01	0.40	0.55
	MID	3	4	326.91	5.02	0.03	0.90	-159.08	-3.09	4.02	0.00	0.38	0.45
	HID	5	4	326.95	5.06	0.03	0.93	-159.10	-9.11	10.83	0.00	0.38	0.81
	LID	2	4	326.97	5.08	0.03	0.97	-159.11	-2.70	2.54	0.00	0.38	-0.10
	Dev	10	4	326.97	5.09	0.03	1.00	-159.12	-9.11	10.83	0.00	0.38	0.81
N	**Forest**	**4**	**4**	**85.51**	**0.00**	**0.35**	**0.35**	**-38.38**	**-0.31**	**0.00**	**0.07**	**0.26**	**-0.15**

	null	1	3	86.87	1.36	0.17	0.52	-40.22	2.47	2.78	0.00	0.14	2.63
	not_Forest	8	4	88.80	3.29	0.07	0.59	-40.03	-0.34	0.18	0.01	0.12	-0.08
	Ag	6	4	88.94	3.44	0.06	0.65	-40.10	-0.14	0.09	0.00	0.13	-0.03
	ISC	7	4	88.96	3.45	0.06	0.71	-40.11	-0.59	0.37	0.00	0.13	-0.11
	OD	9	4	89.05	3.55	0.06	0.77	-40.16	-0.24	0.17	0.00	0.13	-0.04
	HID	5	4	89.06	3.55	0.06	0.83	-40.16	-1.50	1.07	0.00	0.13	-0.22
	MID	3	4	89.07	3.57	0.06	0.89	-40.17	-0.53	0.39	0.00	0.13	-0.07
	Dev	10	4	89.09	3.58	0.06	0.94	-40.17	-0.53	0.40	0.00	0.13	-0.07
	LID	2	4	89.10	3.60	0.06	1.00	-40.18	-0.38	0.29	0.00	0.13	-0.04
P	**HID**	**5**	**4**	**-189.56**	**0.00**	**0.51**	**0.51**	**99.06**	**0.20**	**0.41**	**0.24**	**0.31**	**0.29**
	MID	**3**	**4**	**-188.07**	**1.50**	**0.24**	**0.75**	**98.31**	**0.05**	**0.15**	**0.21**	**0.34**	**0.10**
	ISC	**7**	**4**	**-187.47**	**2.10**	**0.18**	**0.93**	**98.02**	**0.06**	**0.16**	**0.20**	**0.35**	**0.11**
	Dev	**10**	**4**	**-184.31**	**5.25**	**0.04**	**0.97**	**96.44**	**0.04**	**0.12**	**0.16**	**0.31**	**0.08**
	OD	**9**	**4**	**-182.33**	**7.24**	**0.01**	**0.98**	**95.44**	**0.02**	**0.06**	**0.18**	**0.24**	**0.04**
	LID	**2**	**4**	**-182.08**	**7.48**	**0.01**	**0.99**	**95.32**	**0.02**	**0.08**	**0.13**	**0.30**	**0.05**
	Forest	**4**	**4**	**-180.01**	**9.56**	**0.00**	**1.00**	**94.28**	**-0.04**	**-0.01**	**0.10**	**0.27**	**-0.03**
	not_Forest	**8**	**4**	**-179.94**	**9.63**	**0.00**	**1.00**	**94.25**	**0.01**	**0.07**	**0.12**	**0.24**	**0.04**
	null	1	3	-174.50	15.07	0.00	1.00	90.41	0.14	0.19	0.00	0.24	0.16
	Ag	6	4	-172.27	17.30	0.00	1.00	90.42	-0.02	0.02	0.00	0.24	0.00
C:N	**Forest**	**4**	**4**	**276.08**	**0**	**0.35**	**0.35**	**-133.67**	**0.06**	**1.54**	**0.07**	**0.3**	**0.80**
	OD	9	4	277.82	1.74	0.15	0.49	-134.54	-1.82	0.17	0.06	0.24	-0.82
	Ag	6	4	277.88	1.8	0.14	0.63	-134.57	-0.10	1.00	0.05	0.17	0.46
	Null	1	3	278.19	2.11	0.12	0.75	-135.88	15.56	17.23	0.00	0.19	16.36
	ISC	7	4	279.94	3.86	0.05	0.8	-135.6	-1.59	3.37	0.01	0.15	0.94
	not_Forest	8	4	280.28	4.2	0.04	0.85	-135.77	-1.01	1.562	0.00	0.17	0.30
	MID	3	4	280.45	4.38	0.04	0.89	-135.86	-2.10	2.53	0.00	0.19	0.23
	LID	2	4	280.48	4.41	0.04	0.92	-135.87	-1.78	1.61	0.00	0.19	-0.08
C:P	**HID**	**5**	**4**	**792.68**	**0.00**	**0.31**	**0.31**	**-391.96**	**-1505.07**	**-441.54**	**0.19**	**0.19**	**-973.31**

	Dev	**10**	**4**	**794.03**	**1.35**	**0.16**	**0.47**	**-392.64**	**-523.64**	**-137.72**	**0.17**	**0.17**	**-330.68**
	OD	**9**	**4**	**794.26**	**1.58**	**0.14**	**0.61**	**-392.75**	**-201.39**	**-51.84**	**0.17**	**0.17**	**-126.61**
	LID	**2**	**4**	**794.44**	**1.76**	**0.13**	**0.73**	**-392.84**	**-373.39**	**-94.46**	**0.16**	**0.16**	**-233.93**
	MID	**3**	**4**	**794.63**	**1.96**	**0.12**	**0.85**	**-392.94**	**-509.73**	**-126.42**	**0.16**	**0.16**	**-318.07**
	Forest	**4**	**4**	**795.21**	**2.53**	**0.09**	**0.94**	**-393.23**	**40.20**	**172.59**	**0.15**	**0.15**	**106.39**
	ISC	**7**	**4**	**796.43**	**3.75**	**0.05**	**0.99**	**-393.84**	**-497.29**	**-91.90**	**0.13**	**0.15**	**-293.18**
	not_Forest	**8**	**4**	**799.38**	**6.71**	**0.01**	**1.00**	**-395.31**	**-224.18**	**-18.37**	**0.09**	**0.09**	**-121.27**
	null	1	3	801.9	9.23	0.00	1.00	-397.73	576.87	719.00	0.00	0.07	644.63
	Ag	6	4	804.17	11.5	0.00	1.00	-397.71	-46.76	57.96	0.00	0.08	5.02
N:P	**HID**	**5**	**4**	**445.47**	**0.00**	**0.37**	**0.37**	**-218.36**	**-88.44**	**-30.32**	**0.25**	**0.35**	**-59.45**
	MID	**3**	**4**	**446.57**	**1.10**	**0.21**	**0.58**	**-218.91**	**-30.22**	**-9.80**	**0.22**	**0.40**	**-20.05**
	ISC	**7**	**4**	**446.64**	**1.17**	**0.20**	**0.79**	**-218.94**	**-31.89**	**-10.26**	**0.24**	**0.42**	**-21.02**
	Dev	**10**	**4**	**447.53**	**2.06**	**0.13**	**0.92**	**-219.39**	**-30.15**	**-9.23**	**0.21**	**0.37**	**-19.77**
	LID	**2**	**4**	**448.74**	**3.27**	**0.07**	**0.99**	**-219.99**	**-21.23**	**-5.93**	**0.20**	**0.36**	**-13.65**
	not_Forest	**8**	**4**	**454.59**	**9.13**	**0.00**	**0.99**	**-222.92**	**-13.46**	**-1.11**	**0.11**	**0.32**	**-7.30**
	OD	**9**	**4**	**455.00**	**9.53**	**0.00**	**1.00**	**-223.12**	**-10.61**	**-0.67**	**0.12**	**0.27**	**-5.76**
	Forest	**4**	**4**	**455.08**	**9.61**	**0.00**	**1.00**	**-223.16**	**0.43**	**7.60**	**0.07**	**0.43**	**4.02**
	null	1	3	457.54	12.07	0.00	1.00	-225.55	35.43	44.62	0.00	0.34	39.87
	Ag	6	4	459.67	14.20	0.00	1.00	-225.46	-3.71	2.35	0.00	0.34	-0.64
Cd	**ISC**	**7**	**4**	**1281.43**	**0.00**	**0.47**	**0.47**	**-118.35**	**0.69**	**2.78**	**0.15**	**0.51**	**1.70**
	MID	**3**	**4**	**1281.81**	**0.38**	**0.39**	**0.86**	**-118.54**	**0.60**	**2.55**	**0.14**	**0.54**	**1.52**
	HID	**5**	**4**	**1285.46**	**4.03**	**0.06**	**0.93**	**-120.36**	**0.83**	**6.30**	**0.09**	**0.50**	**3.51**
	Dev	**10**	**4**	**1286.54**	**5.11**	**0.04**	**0.96**	**-120.90**	**0.16**	**1.90**	**0.08**	**0.48**	**1.00**
	LID	**2**	**4**	**1288.10**	**6.67**	**0.02**	**0.98**	**-121.68**	**0.00**	**1.17**	**0.05**	**0.46**	**0.57**
	null	1	3	1289.76	8.33	0.01	0.99	-123.63	0.91	1.80	0.00	0.39	1.35
	not_Forest	8	4	1291.06	9.63	0.00	0.99	-123.16	-0.30	0.88	0.02	0.41	0.29
	OD	9	4	1291.69	10.26	0.00	0.99	-123.48	-0.32	0.60	0.01	0.41	0.13
	Forest	4	4	1291.73	10.30	0.00	1.00	-123.50	-0.25	0.42	0.00	0.38	0.09

	Ag	6	4	1291.90	10.47	0.00	1.00	-123.58	-0.37	0.27	0.00	0.39	-0.05
Na	ISC	7	4	1352.01	0.00	0.19	0.19	-671.72	-264.58	3102.22	0.05	0.63	1498.60
	Null	1	3	1352.60	0.60	0.14	0.33	-673.14	2174.77	3741.21	0.00	0.72	2957.90
	MID	3	4	1352.61	0.61	0.14	0.47	-672.03	-391.25	2599.69	0.04	0.64	1168.10
	HID	5	4	1352.81	0.80	0.13	0.60	-672.12	-1168.27	6902.66	0.03	0.65	2985.80
	Dev	10	4	1353.08	1.07	0.11	0.71	-672.26	-464.64	2194.32	0.03	0.67	914.80
	LID	2	4	1353.34	1.33	0.10	0.81	-672.39	-364.04	1438.22	0.02	0.68	568.80
	not_Forest	8	4	1354.54	2.53	0.05	0.86	-672.99	-628.35	1093.39	0.00	0.71	237.90
	Ag	6	4	1354.81	2.81	0.05	0.91	-673.12	-471.73	565.54	0.00	0.72	38.26
	Forest	4	4	1354.83	2.82	0.05	0.95	-673.13	-454.87	426.85	0.00	0.72	-11.81
	OD	9	4	1354.83	2.83	0.05	1.00	-673.14	-666.84	655.43	0.00	0.72	-4.52
	ISC+								-372.17	2766.05			1262.2
	Lat		**5**					-668.58	**147.59**	**1180.38**	**0.19**	**0.65**	**648.0**
Zn	**Forest**	4	4	853.23	0.00	0.53	0.53	-422.33	6.58	39.20	0.06	0.71	22.95
	MID	3	4	855.94	2.71	0.14	0.67	-423.69	5.25	107.17	0.06	0.65	56.34
	HID	5	4	856.84	3.61	0.09	0.76	-424.14	-1.91	274.57	0.05	0.64	136.99
	ISC	7	4	857.40	4.17	0.07	0.82	-424.42	-5.46	111.01	0.04	0.65	52.96
	Dev	10	4	858.14	4.92	0.05	0.87	-424.79	-9.92	84.87	0.03	0.65	37.49
	null	1	3	858.35	5.12	0.04	0.91	-426.01	122.11	178.72	0.00	0.65	150.28
	LID	2	4	858.97	5.75	0.03	0.94	-425.20	-11.77	53.60	0.02	0.65	20.88
	Ag	6	4	859.03	5.81	0.03	0.97	-425.24	-30.28	7.12	0.03	0.64	-11.77
	OD	9	4	860.27	7.04	0.02	0.99	-425.85	-18.53	32.55	0.01	0.64	7.17
	not_Forest	8	4	860.56	7.33	0.01	1.00	-426.00	-30.15	34.78	0.00	0.65	2.35

Table 5.3. Mean percent land use cover and mean latitude (Lat) in decimal degrees, standard deviation (SD), minimum (min), maximum (max) and the transformation factor (T. factor, equal to one SD of arcsine-transformed land use) for expressing regression coefficients in terms of percent land cover. For example: in the x-axis scales in Figure 2, 1 unit equates to a 19.4% change in Forest, a 3.6% change in ISC, etc. Land use codes are as follows, Ag = Agriculture, OD = Open Development, LID = Low Intensity Development, Forest = Forest Cover, ISC = impervious surface cover, MID = Medium Intensity Development, HID = High Intensity Development, Dev = LID+MID+HID, not_Forest = Ag + Dev.

land use	mean	SD	min	max	T. factor
Forest	76.3	12.6	13.0	98.9	19.4
ISC	1.4	3.5	0.0	34.2	3.6
OD	6.1	3.8	0.6	27.2	3.9
LID	1.7	3.1	0.0	26.1	3.1
MID	0.7	1.8	0.0	17.2	1.8
HID	0.3	1.5	0.0	14.8	1.5
Dev	2.7	6.3	0.0	58.1	6.6
Ag	2.1	3.8	<0.1	25.3	3.8
not_Forest	10.9	10.2	0.6	85.3	11.5
Latitude	36.1	2.5	33.0	44.0	na

Table 5.4. Metal concentration of cadmium (Cd), sodium (Na), and zinc (Zn) in µg g^{-1} (ppm) of *Podostemum ceratophyllum* stem and leaf tissue.

	Cd	Na	Zn
Mean	1.17	2898.90	137.06
Min	0.36	145.08	40.08
Median	0.84	2535.23	117.37
Max	10.61	11990.58	386.70

Figures for 15N and metals MS

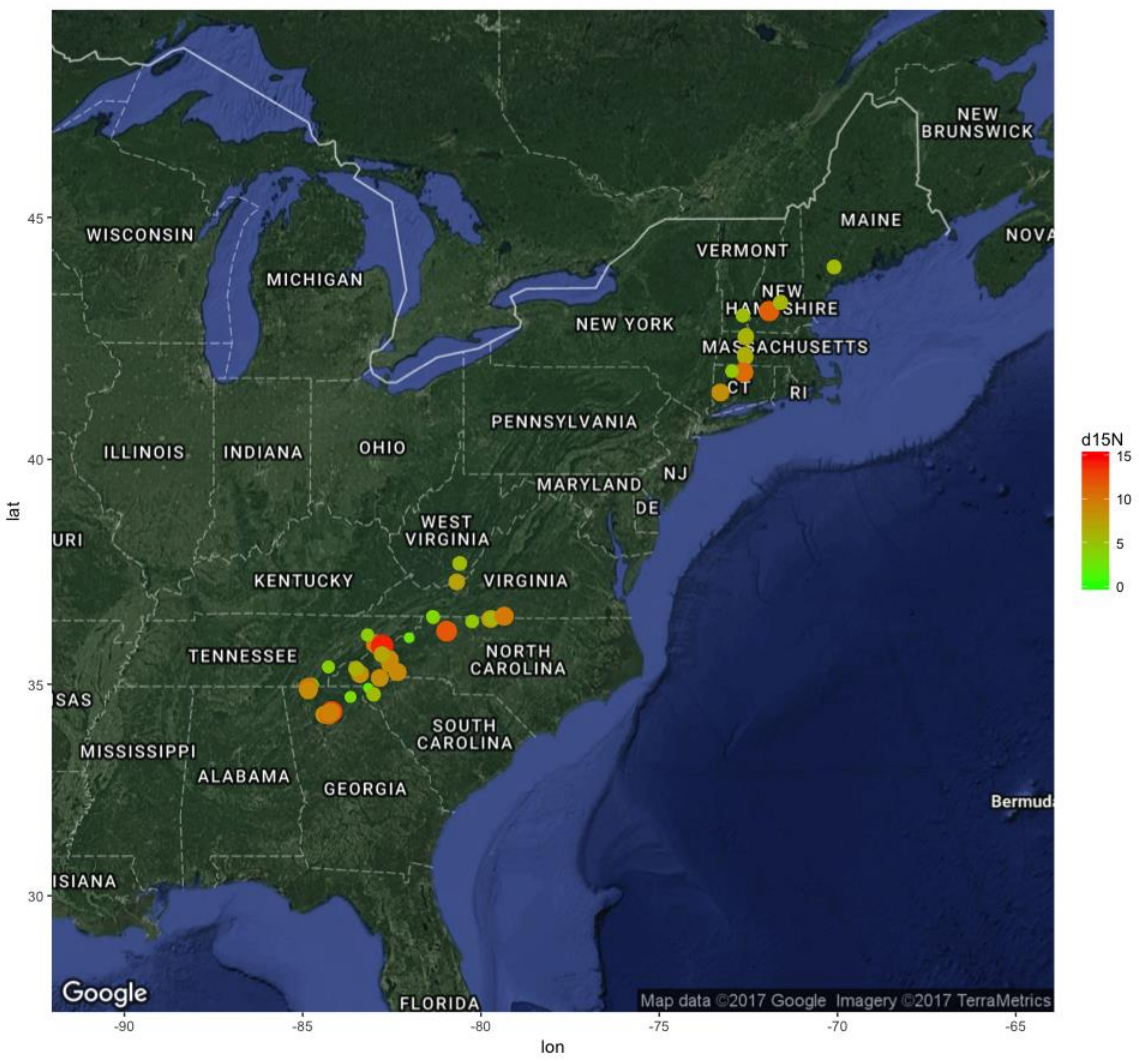

Figure 5.1. Between 2013 and 2015 samples of *Podostemum ceratophyllum* were collected from 59 locations between the Piedmont of Georgia and southern Maine. Smaller brighter green circles indicate lower concentrations of δ^{15}N, and larger, redder circles indicate higher concentrations of δ^{15}N, an isotope of nitrogen correlated with sewage and septic inputs and animal manure. Mean δ^{15}N = 7.06

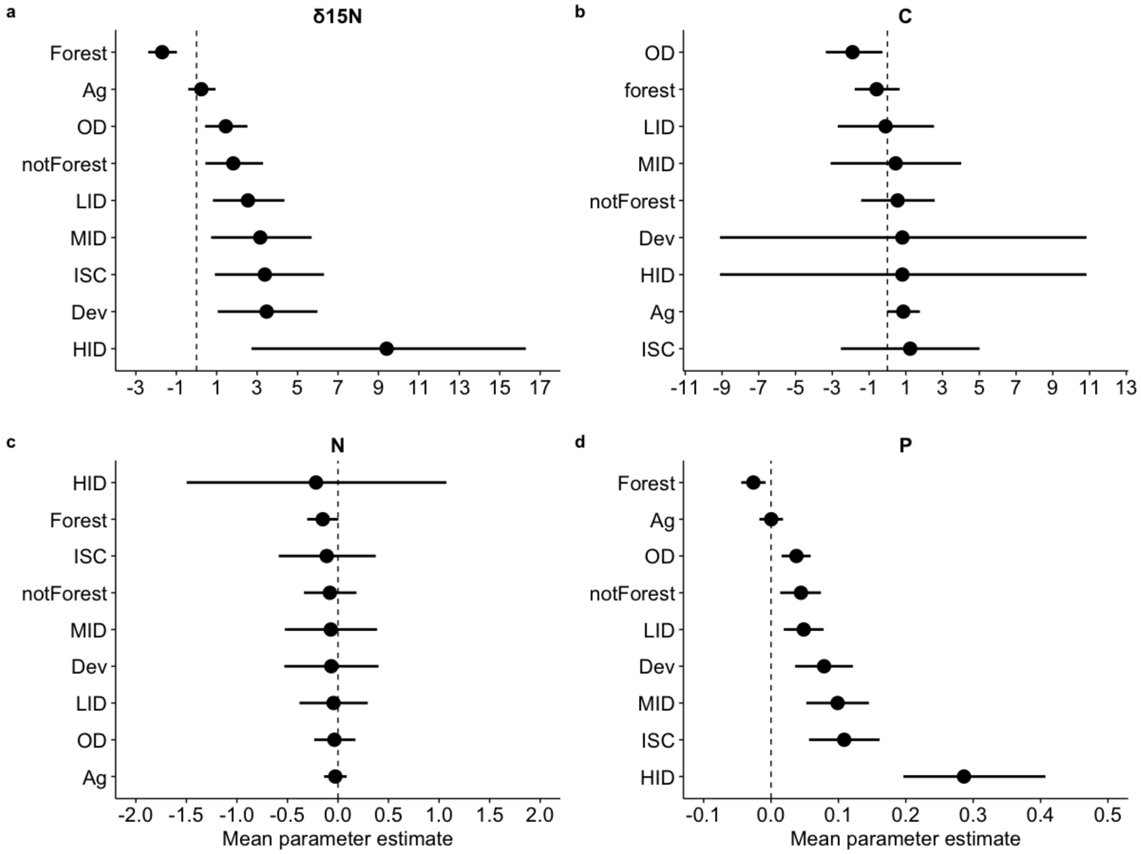

Figure 5.2. Plotted values show the mean (and 95% confidence interval) regression coefficient for each land use type as a predictor of *Podostemum* composition. The x-axes are scaled relative to standard deviations of the arcsine-transformed land use percentages. Confidence intervals that do not cross 0 can be considered significant at *a* = 0.05 level. Land use codes are as follows, Ag = Agriculture, OD = Open Development, LID = Low Intensity Development, Forest = Forest Cover, ISC = impervious surface cover, MID = Medium Intensity Development, HID = High Intensity Development, Dev = LID+MID+HID, notForest = Ag + Dev.

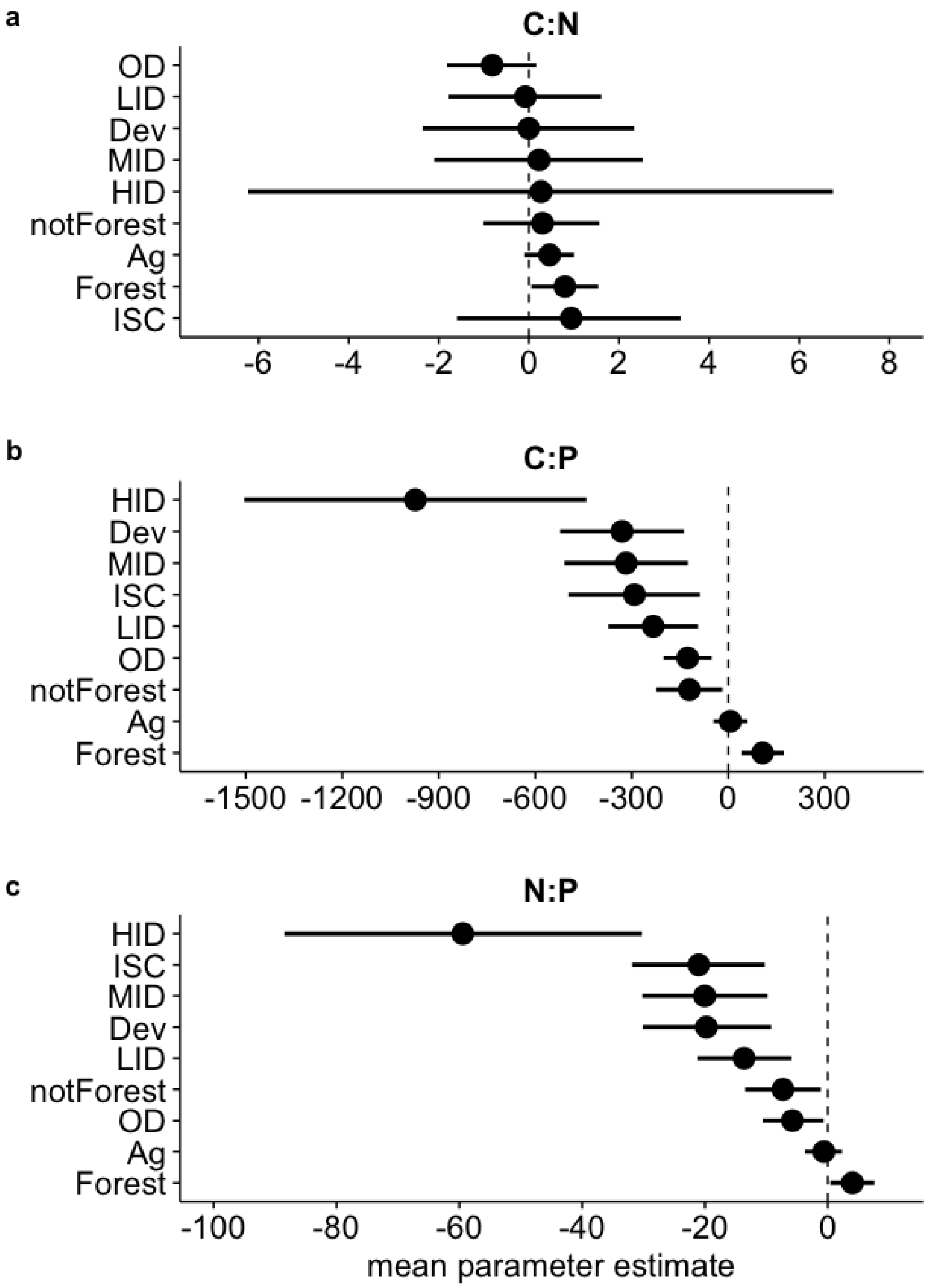

Figure 5.3. Plotted values show the mean (and 95% confidence interval) regression coefficient for each land use type as a predictor of *Podostemum* composition. The x-axes are scaled relative to standard deviations of the arcsine-transformed land use percentages. Confidence intervals that do not cross 0 can be considered significant at $a = 0.05$ level. Land use codes are as follows, Ag = Agriculture, OD = Open Development, LID = Low Intensity Development, Forest = Forest Cover, ISC = impervious surface cover, MID = Medium Intensity Development, HID = High Intensity Development, Dev = LID+MID+HID, notForest = Ag + Dev

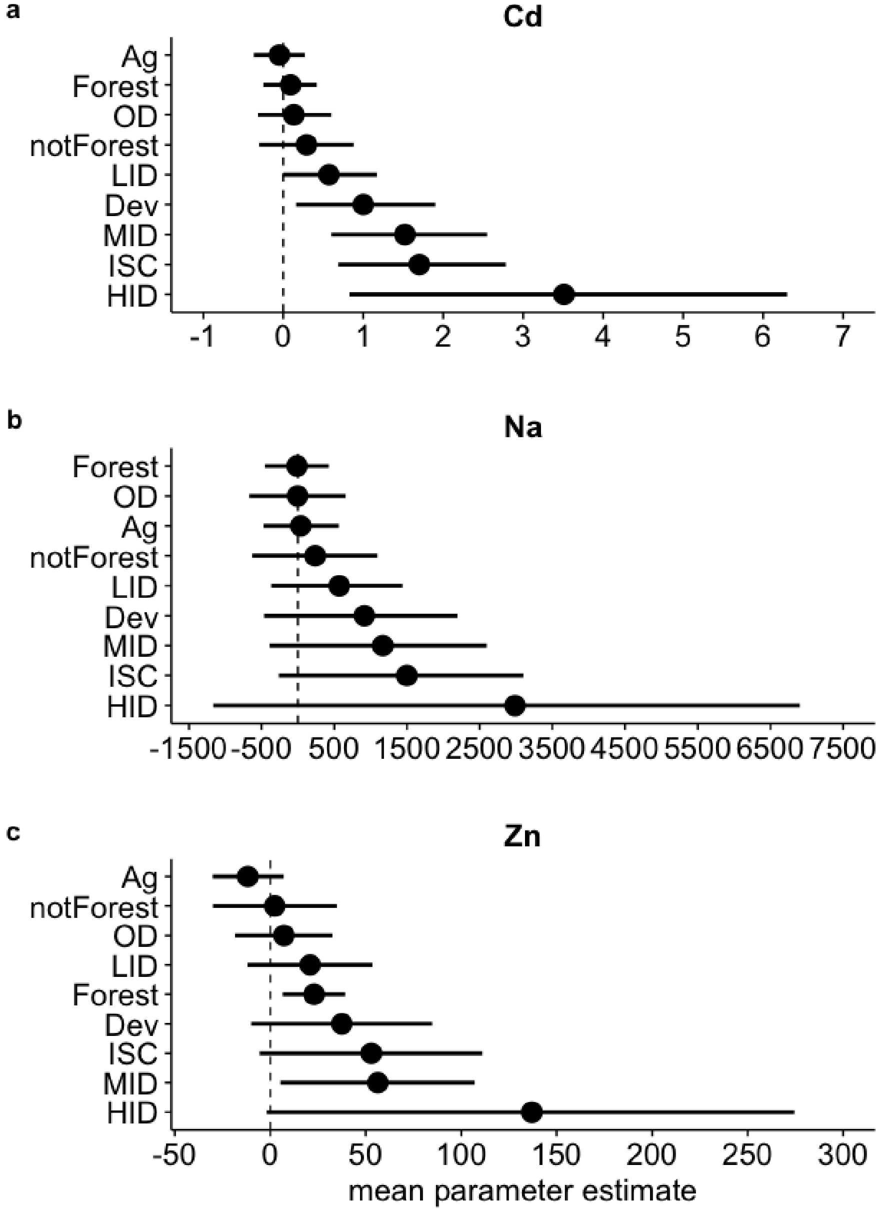

Figure 5.4. Plotted values show the mean (and 95% confidence interval) regression coefficient for each land use type as a predictor of *Podostemum* composition. The x-axes are scaled relative to standard deviations of the arcsine-transformed land use percentages. Confidence intervals that do not cross 0 can be considered significant at *a* = 0.05 level. Land use codes are as follows, Ag = Agriculture, OD = Open Development, LID = Low Intensity Development, Forest = Forest Cover, ISC = impervious surface cover, MID = Medium Intensity Development, HID = High Intensity Development, Dev = LID+MID+HID, nForest = Ag + Dev.

CHAPTER 6

CONCLUSION

Preservation of freshwater resources is necessary to protect human health and there is an urgency to better understand how anthropogenic activities (urbanization, agriculture, flow alteration, nutrient enrichment) affect freshwater resources to minimize possibly irreversible negative impacts. Anthropogenic nutrient enrichment of freshwaters has negative consequences for human health and ecosystem services (Zurawell et al., 2005; Paerl et al., 2011; Paerl et al., 2016) and flow alteration is a significant cause of decline and extinction of freshwater fauna (Xenopoulos and Lodge, 2006; Poff et al., 2009; Strayer and Dudgeon, 2010). Additionally, flow alteration and impoundments can negatively impact major economic and dietary resources for local human populations (Ziv et al., 2012), and flow alteration can negatively affect secondary production (Kennedy et al., 2016), reducing subsidies available to adjacent terrestrial systems (Baxter et al., 2005). Furthermore, the conservation of freshwater systems is necessary on a regional scale for the preservation and restoration of downstream estuaries and marine ecosystems (Sklar and Browder, 1998; Freeman et al., 2007). Therefore, we have focused our research on the influence of nutrients, flow alteration and land use in lotic systems by investigating microbial metabolic rates, nutritional quality, and toxicity of basal resources in large streams and rivers in eastern North America using local, landscape and large regional scale studies.

First we explored the influence of nutrients on both autotrophic and heterotrophic biofilms across a large watershed in the Southern Appalachian landscape, using both functional and structural metrics to better understand the state of nutrient limitation in streams already experiencing watershed modification and enrichment. Next, we examined the ecologically important flowering aquatic plant, *Podostemum ceratophyllum* Michx., henceforth referred to as *Podostemum*, which grows in abundance in rivers across eastern North America where it attaches to stable substrates in fast flowing water with high light. We investigated how variation in water velocity on the local scale (a few meters) influences herbivory pressure. Our study provided insight into environmental factors influencing the plant's accrual of biomass, how the plant interacts with consumers, and factors controlling the distribution of the *Podostemum* within the channel. Lastly, we explored the influence of land use on basal resource quality and toxicity in eastern North America using *Podostemum* as a model organism. Our sampling locations ranged from the piedmont of Georgia up through the Appalachian Mountains and into southern Maine. We presented evidence that the elemental composition of *Podostemum* is reflective of land use in the watershed and explored changes in nutrient quality.

By utilizing multiple scales of investigation and novel research questions, we shed new light on the ways in which components of eastern lotic systems are responding to anthropogenic influences and elucidated mechanisms behind changes in basal resource quality, quantity, and toxicity in large streams and rivers in eastern North America.

Biofilms are nutrient limited across an eastern montane landscape

In our analysis of nutrient limitation of autotrophic and heterotrophic biofilms in the Southern Appalachian Mountains landscape, we found that nutrient limitation was in part dependent on carbon availability and light, and that both phosphorus (P) and nitrogen (N) can limit primary production in freshwater systems. Our best supported models for predicting GPP, CR and algal biomass all included canopy cover and our study provided additional evidence that high density canopy cover (above 80%) is important for managing algal blooms in 2nd-3rd order streams as they become enriched with nutrients. Our results indicated that, across the landscape, GPP was primarily limited by P, similar to findings by Johnson et al. (2009) who also found phosphorus limitation of GPP. Likewise, we found algal biomass was strongly co-limited (N+P), but the range of *in situ* conditions experienced by our field study provides additional support for a strong effect of canopy cover on algal biomass (Mosisch et al., 2001). Conversely, agricultural streams may support little algal biomass under conditions of low canopy cover and high nutrient availability because of high sedimentation rates, which can increase scour and remove algal biomass. This noise in predicting the influence of canopy is evidenced by our top model for predicting algal biomass (P treatment, $R^2= 0.17$), which indicates a positive correlation between algal biomass and increasing water column DIN and SRP, and included canopy cover, however, confidence intervals for canopy substantially crossed zero.

Similarly, we found that canopy cover was included in all AICc top models for community respiration on labile organic carbon substrates, however we did not find substantial evidence for nutrient limitation after approximately 20 days on organic substrates when substrates were not protected from the effects of consumers and scour. However, we did observe an effect of water column nutrients in our consumer exclosure experiments, indicating that consumer grazing may interact with increased nutrient availability to increase basal carbon loss from streams. Taken together, these two major findings (increased storage of autotrophic biomass and decreased storage of labile carbon via increased heterotrophic activity) indicate that increasing nutrient availability can change basal resources available to consumers. These shifts in resources are expected to affect functional feeding groups in different ways. For example, resources for shredders are anticipated to decrease due to decreasing leaf material available for ingestion, while resources for scrapers are anticipated to increase because of increased algal biomass. However, shifts in biofilm community composition may also have large effects on how changes in resources affect consumers. For example, cyanobacteria can become prominent in streams with high nutrient concentrations and increased water temperature (Paerl and Huisman, 2008), but cyanobacteria can be toxic to consumers (Zurawell et al., 2005) and elicit changes in the food web dynamics. Our results provide insight into how changes in nutrients and carbon availability can influence metabolic pathways in freshwaters, which indicate changes in carbon storage and release from freshwater systems.

The influence of water velocity on trophic interactions

Information on *Podostemum* response to environmental changes is necessary to conserve and restore the species, and our study on the effect of water velocity on herbivory of *Podostemum* provides new insight into the ecology of the species. We found that water velocity exerted a top down pressure on herbivory, such that herbivory appeared to be severely reduced when water velocity was greater than approximately 0.5

m s^{-1}. For example, under conditions of low water velocity (~<0.05 ms^{-1}), we found that *Podostemum* is rapidly consumed. Thus, changes in water velocity altered benthic resource storage and flux, which in turn is expected to influence benthic habitat complexity (Hutchens et al., 2004). Therefore, there is a compelling need to better understand the ecological implications of flow management on river systems because management decisions can reduce (Freeman et al., 2001), eliminate, or otherwise affect components of the benthic community (Poff et al., 2009; Kennedy et al., 2016). In many cases, managing for flow regimes that more closely mimic natural flow regimes in the eastern U.S. has the potential to restore *Podostemum* habitat in rivers where the plant has been extirpated (Philbrick and Crow, 1983; Wood and Freeeman, 2017), and restoration of *Podostemum* may increase macroinvertebrate biomass (Nelson and Scott, 1962; Grubaugh and Wallace, 1995) and fish abundance (Argentina et al., 2010). Management for and the restoration of *Podostemum* may also help mitigate the occurrence of algal blooms by removing nutrients from the water column ~~thus reducing water column nutrients~~, although this theory has not been thoroughly investigated. Because the family of *Podostemum*, Podostemaceae, is a globally distributed group with most members occupying similar swift-water habitats (Novelo and Philbrick, 1997; Philbrick and Novelo, 2004; Philbrick et al., 2010), other species in the family may be useful to river managers and aquatic ecologists by providing novel assessment tools to better predict the effects of flow management on river systems.

Land use is reflected in the elemental composition of Podostemum

After analyzing *Podostemum* collected from across a large portion of the plant's range, we concluded that land use is reflected in the elemental composition of the plant. Our results indicated terrestrial land use is contributing to an increase in elements commonly associated with urbanization (i.e., Cd, N, P, Zn) in *Podostemum*, and our results supported the conclusion of Xia et al. (2014) that P can accumulate in freshwater systems faster than N, at least in freshwater macrophytes. Furthermore, our results showed that *Podostemum* provides consumer resources of comparable quality compared with other submerged macrophytes (Bakker et al., 2016) but we also presented evidence that elements such as Cd and Zn may be accumulating in plant tissue. We also found that increasing Na concentration in the plant is correlated with increasing latitude, supportive of other studies (Kaushal et al., 2005) (Daley et al., 2009) that have found that water in many rivers in the northeast are experiencing highly elevated and potentially harmful salinity concentrations.

We also showed that urbanization and the loss of forest cover was correlated with changes in basal resource quality, and our data raised concerns about the possibility of trace metal loading in benthic food webs as a result of large scale watershed urbanization. Because urbanization is expanding in much of *Podostemum*'s range, utilizing the plant to help quantify the effects of land use appears warranted. Additionally, because the family Podostemaceae is globally distributed, investigations into the utilization of others species in the family as indicators of land use and changes in benthic resources appears viable. At present, the major stressors causing declines in *Podostemum* abundance in North America are poorly understood but our data indicated that loss of the plant from river systems could alter benthic food webs and the flow of elements through the ecosystem.

In total, the results of these studies help illustrate the wide-ranging impacts of anthropogenic actions on freshwater systems. We found that nutrients can alter basal resources in large streams, increasing some pools while decreasing others, and these effects are expected to influence the pathways in which resources flow through aquatic food webs. We also found that land use influences basal resource quality and toxicity, and that water velocity can regulate the movement of these resources into the food web. Our findings provide new insight into the influence of land use, nutrient enrichment and water velocity on basal resources in large streams and rivers, and contribute to the growing body of research linking anthropogenic activity to changes in freshwater resources.

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APPENDIX A. Chapter 2. Percent land use and impervious surface (ISP), watershed size, mean in stream algal biomass (Chl *a*) collected from rock scrapings, stream water nutrients, molar N:P ratio, discharge and water temperature by stream.

	Land cover								Measured Response Variables					
Watershed	%Urban	%Forest	%Grass -land	%Pasture /Crop	% ISC	Water- shed Size	Chl a	N-NH4	N-NO3	DIN	SRP	N:P	Q_LS^{-1}	Temp ºC
Blacks	10.1	77.8	0.9	9.1	8.1	41.0	27.2	25.1	352.4	377.5	12.9	64.6	19.2	20.3
Cowee	3.1	93.1	1.0	1.5	2.9	26.7	13.8	7.3	56.0	63.3	6.8	20.5	266.6	21.2
Caler	5.2	89.8	1.4	2.3	3.6	16.8	19.8	10.1	54.6	64.7	11.9	12.1	174.0	19.9
Craw	9.3	85.3	0.8	2.4	9.1	0.5	1.4	30.5	446.8	477.4	5.5	191.2	2.3	18.1
Dalton	0.4	98.8	0.0	0.0	0.5	13.5	4.0	7.6	40.3	47.8	8.9	11.9	19.7	18.1
Darnel	2.7	96.7	0.2	0.0	0.8	2.8	31.1	9.4	115.9	125.4	7.1	38.9	182.5	18.6
Frog	48.3	43.1	1.6	5.0	45.1	4.9	24.7	35.5	413.8	449.3	9.0	110.9	30.7	20.8
Howard	1.1	97.0	0.0	0.0	0.8	0.8	0.7	64.3	197.2	261.5	11.3	51.1	19.6	17.7
Jerry	14.8	64.9	1.5	15.6	13.5	1.5	12.5	34.5	432.0	466.5	17.2	60.2	10.0	20.1
Jones	3.2	93.9	0.7	1.6	2.3	15.5	16.9	10.6	73.5	84.1	14.9	12.5	171.8	19.9
Mica	1.5	97.8	0.5	0.2	0.3	3.2	2.2	5.2	45.1	50.3	12.5	8.9	NA	17.9
Shop	2.1	97.1	0.0	0.0	2.1	15.7	5.8	4.4	51.8	56.1	9.1	13.7	115.6	17.9
Skee	6.0	88.5	2.0	1.8	3.9	6.2	11.2	20.1	183.2	203.4	5.8	77.2	47.2	19.6
Tess	3.7	91.1	0.9	1.4	3.0	38.7	33.1	42.9	605.4	648.3	9.6	150.1	374.6	20.8
Watauga	13.0	81.0	0.9	3.4	13.7	16.7	26.1	11.6	129.4	141.0	7.8	40.1	74.1	20.8

APPENDIX B. Chapter 4: Jags model code

The following R and JAGS code was used to estimate mean stem growth rate and mean percent of stem growth that was consumed, daily, during a 77-d consumer exclusion versus consumer access experiment. Data were means of 10 stem lengths measured on each date, in exclosures that were either electrified (treatment) or not (control), and listed in a data frame ("pod.expt", below). The model was run for 300,000 iterations after a burn-in of 100,000, thinned by 4 and using 3 chains. Convergence was assessed satisfactory if R-hat was less than 1.1.

```
# Set up files with data and covariates
nplots<-5  # number of paired experimental plots
pairs<-2  # 2 units per plot (control and treatment)
ndates<-12 # number of dates including NAs
names(pod.expt)<-c("Treatment", "Stem.Length_Avg_per_plot", "Time_day",
"Plot","Plot_Pair")
length<-array( , dim=c(nplots,ndates, pairs))
length[,,]<-as.array(pod.expt$Stem.Length_Avg_per_plot)  # length data
# indicator variable for control (1) or treatment (0) for each length measurement
Control<-array( ,dim=c(nplots, pairs))
Control[,1]<-1
Control[,2]<-0
# vector of the elapsed days for each date
days<-c(0,7,14,21,28,35,42,49,56,63,70,77)
# JAGS model to estimate growth and consumption rates
sink("Pod_growth_and_consumption.jags")
cat("
   model {

   # Specify priors
   for (i in 1:nplots){
   for (k in 1:pairs){
   # model unit-specific r as mean growth rate - consumption rate
   # growth rate is plot specific with a mean = alpha.int
   # consumption rate is 0 if a treatment unit, and a proportion (alpha.control) of growth
in control units
   r[i,k]<-g[i,k]-c[i,k]
   g[i,k]<-alpha.int +epsilon[i]
   c[i,k]<-alpha.control*g[i,k]*Control[i,k]
   }}
   sigma.obs~dunif(0,1)  #std dev of observation error; prior is 0 to 1cm
   tau.obs<-pow(sigma.obs, -2)  # precision, observation error
   sigma2.obs<-pow(sigma.obs,2) # variance, observation error
   sigma.plots~dunif(0,1)  # std dev on growth rates among plots, prior is 0 to 1 d-1
   tau.plots<-pow(sigma.plots, -2)
   sigma2.plots<-pow(sigma.plots,2)
```

```
   alpha.int~dunif(0,0.5)  # mean growth rate; prior is 0 to 0.5 d-1
   alpha.control~dunif(0.01,0.99)  # consumption rate as proportion of growth; prior is 1
to 99%
   for (i in 1:nplots){
         epsilon[i]~dnorm(0, tau.plots)  # random variation, growth rates among plots
   for (k in 1:pairs){
         initial.length[i,k]~dunif(0.3,0.8)  # mean initial stem length, each unit
         length[i,1,k] ~dnorm(initial.length[i,k], tau.obs)
   }}
#ecological process model
   for (i in 1:nplots){
         for (j in 2:ndates){
                  for (k in 1:pairs){
                           length[i,j,k] ~dnorm(mean.length[i,j,k], tau.obs)
                           mean.length[i,j,k]<-initial.length[i,k]*(exp(r[i,k]*days[j]))
   }}}
}
   }
   ",fill = TRUE)
sink()
# data vectors
win.data <- list(length = length, nplots=nplots, pairs=pairs, ndates=ndates,
Control=Control,  days=days)
# initial values for the unknowns
inits <- function(){list(alpha.int=runif(1,0,.1), sigma.obs=runif(1,0,1),
alpha.control=runif(1,0.3,0.99), sigma.plots=runif(1,0,0.1))}
# parameters to monitor
params<-c("alpha.int", "alpha.control", "sigma2.obs", "sigma2.plots")
# MCMC settings
ni <- 400000
nt <- 4
nb <- 100000
nc <- 3
```

“pod.expt” data:

Treatment	Stem Length_Avg_per_plot	Time_day	Plot	Plot_Pair
Control	0.57	0	1a	1
Control	0.33	0	2a	2
Control	0.35	0	3b	3
Control	0.54	0	4a	4
Control	0.74	0	5a	5
Control	0.79	7	1a	1
Control	0.63	7	2a	2
Control	0.46	7	3b	3
Control	0.57	7	4a	4

Control	0.59	7	5a	5
Control	0.68	14	1a	1
Control	0.6	14	2a	2
Control	0.7	14	3b	3
Control	0.74	14	4a	4
Control	0.77	14	5a	5
Control	0.71	21	1a	1
Control	0.68	21	2a	2
Control	0.46	21	3b	3
Control	0.67	21	4a	4
Control	0.73	21	5a	5
Control	0.56	28	1a	1
Control	0.45	28	2a	2
Control	0.67	28	3b	3
Control	0.52	28	4a	4
Control	0.69	28	5a	5
Control	0.56	35	1a	1
Control	0.45	35	2a	2
Control	0.52	35	3b	3
Control	0.48	35	4a	4
Control	0.6	35	5a	5
Control	NA	42	1a	1
Control	NA	42	2a	2
Control	NA	42	3b	3
Control	NA	42	4a	4
Control	NA	42	5a	5
Control	0.59	49	1a	1
Control	0.51	49	2a	2
Control	0.56	49	3b	3
Control	1.08	49	4a	4
Control	0.65	49	5a	5
Control	0.62	56	1a	1
Control	0.51	56	2a	2
Control	0.45	56	3b	3
Control	0.51	56	4a	4
Control	0.63	56	5a	5
Control	0.6	63	1a	1
Control	0.34	63	2a	2
Control	0.45	63	3b	3
Control	0.7	63	4a	4
Control	0.8	63	5a	5
Control	0.64	70	1a	1
Control	0.73	70	2a	2

Control	0.71	70	3b	3
Control	0.63	70	4a	4
Control	0.84	70	5a	5
Control	1.11	77	1a	1
Control	0.94	77	2a	2
Control	0.78	77	3b	3
Control	1.22	77	4a	4
Control	1.22	77	5a	5
Exclosure	0.47	0	1a	1
Exclosure	0.45	0	2a	2
Exclosure	0.49	0	3b	3
Exclosure	0.55	0	4a	4
Exclosure	0.71	0	5a	5
Exclosure	0.71	7	1a	1
Exclosure	0.63	7	2a	2
Exclosure	0.59	7	3b	3
Exclosure	0.5	7	4a	4
Exclosure	1.02	7	5a	5
Exclosure	0.86	14	1a	1
Exclosure	1.16	14	2a	2
Exclosure	0.52	14	3b	3
Exclosure	0.41	14	4a	4
Exclosure	1.57	14	5a	5
Exclosure	1.16	21	1a	1
Exclosure	1.48	21	2a	2
Exclosure	0.52	21	3b	3
Exclosure	0.47	21	4a	4
Exclosure	2.44	21	5a	5
Exclosure	1.01	28	1a	1
Exclosure	1.09	28	2a	2
Exclosure	0.49	28	3b	3
Exclosure	0.33	28	4a	4
Exclosure	2.02	28	5a	5
Exclosure	0.95	35	1a	1
Exclosure	1.22	35	2a	2
Exclosure	0.42	35	3b	3
Exclosure	0.39	35	4a	4
Exclosure	1.81	35	5a	5
Exclosure	NA	42	1a	1
Exclosure	NA	42	2a	2
Exclosure	NA	42	3b	3
Exclosure	NA	42	4a	4
Exclosure	NA	42	5a	5

Exclosure	0.41	49	1a	1
Exclosure	1.58	49	2a	2
Exclosure	0.94	49	3b	3
Exclosure	0.51	49	4a	4
Exclosure	3.11	49	5a	5
Exclosure	0.68	56	1a	1
Exclosure	1.67	56	2a	2
Exclosure	0.55	56	3b	3
Exclosure	0.58	56	4a	4
Exclosure	4.46	56	5a	5
Exclosure	1.78	63	1a	1
Exclosure	2.81	63	2a	2
Exclosure	0.87	63	3b	3
Exclosure	0.61	63	4a	4
Exclosure	5.01	63	5a	5
Exclosure	2.36	70	1a	1
Exclosure	4.68	70	2a	2
Exclosure	1.46	70	3b	3
Exclosure	0.8	70	4a	4
Exclosure	5.85	70	5a	5
Exclosure	4.08	77	1a	1
Exclosure	6.51	77	2a	2
Exclosure	2.5	77	3b	3
Exclosure	2.13	77	4a	4
Exclosure	6.47	77	5a	5

Appendix C. Chapter 5. Site Characteristics, percent aggregated land cover, river name, state, latitude and longitude for each sampling location.

UID	Collection number	River	State	Lat_DD	Long_DD	Forest	OD	LID	MID	HID	ISC	Ag	Dev	not Forest
1	NTOE_1_02_07_14	North Toe River	NC	36.013531	-82.229904	81.57	6.42	0.76	0.32	0.09	0.73	7.62	1.17	15.21
2	Cane_1_02_07_14	Cane Creek	NC	36.013518	-82.191433	81.46	5.31	0.38	0.12	0.02	0.42	9.64	0.52	15.47
3	Wilson_1_03_07_14	Wilson Creek	NC	35.924926	-81.739021	95.73	2.28	0.07	0.00	0.00	0.10	1.04	0.07	3.39
4	Wilson_01_04_07_14	Wilson Creek	NC	35.924926	-81.739021	95.73	2.28	0.07	0.00	0.00	0.10	1.04	0.07	3.39
5	Smith_1_04_07_14	Smith River	NC	36.507069	-79.755464	71.74	5.71	2.31	0.74	0.35	1.79	10.00	3.40	19.11
6	Dan_1_04_07_14	Dan River	NC	36.471336	-79.739223	67.50	5.68	1.49	0.43	0.19	1.19	14.90	2.11	22.69
7	Dan_2_04_07_14	Dan River	NC	36.485822	-79.719248	67.50	5.68	1.50	0.43	0.19	1.19	14.89	2.12	22.69
8	Dan_3_04_07_14	Dan River	NC	36.49235	-79.707956	67.43	5.70	1.51	0.44	0.20	1.21	14.88	2.15	22.73
13	FrBr_1_26_07_14	French Broad	NC	35.462632	-82.54869	70.33	11.95	2.37	0.95	0.37	1.90	12.06	3.69	27.70
14	FrBr_2_26_07_14	French Broad	NC	35.474275	-82.554778	69.88	12.17	2.50	1.04	0.40	2.02	11.98	3.94	28.09
15	FrBr_1_27_07_14	French Broad	NC	35.622664	-82.594522	67.84	14.18	3.44	1.45	0.53	2.78	10.66	5.42	30.26
16	FrBr_2_27_07_14	French Broad	NC	35.682675	-82.615845	66.70	14.29	3.45	1.42	0.51	2.76	11.62	5.38	31.29
17	WQ_C47	Conasauga River	GA	34.7833643	-84.87281506	67.50	5.46	1.11	0.35	0.21	0.94	18.17	1.67	25.30
18	WQ_C13	Conasauga River	GA	34.96173141	-84.78939694	81.37	2.90	0.37	0.08	0.02	0.30	10.82	0.47	14.19
19	WQ_C15	Conasauga River	Ga	34.92140351	-84.84163215	74.99	3.20	0.43	0.11	0.04	0.34	15.19	0.58	18.97
20	WQ_C7	Conasauga River	GA	35.00943	-84.7352	96.17	1.05	0.06	0.01	0.00	0.06	1.50	0.07	2.62
21	WQ_C5	Conasauga River	GA	35.010965	-84.72527	97.09	0.87	0.01	0.00	0.00	0.03	0.95	0.01	1.83
22	WQ_C3	Conasauga River	GA	34.99557171	-84.64299911	98.86	0.61	0.00	0.00	0.00	0.01	0.17	0.00	0.78
23	WQ_E18 (7-29/30-14)	Etowah River	GA	34.37248253	-84.1610965	81.72	6.03	1.21	0.31	0.10	0.79	5.51	1.62	13.16
24	WQ_E31	Etowah River	GA	34.29908243	-84.39663386	75.28	8.64	2.04	0.47	0.15	1.28	7.68	2.66	18.98
26	SFNR_1_14_08_14	South Fork of the New River	NC	36.52744	-81.337934	64.61	7.77	0.82	0.60	0.22	0.92	21.24	1.64	30.65
27	NFNR_1_14_08_14	North Fork of the New River	NC	36.549551	-81.37325	80.24	3.73	0.14	0.07	0.03	0.24	12.55	0.24	16.52
28	Dan_1_15_08_14	Dan River	NC	36.48547	-79.755668	65.64	5.55	1.04	0.25	0.11	0.84	17.27	1.40	24.22

29	Dan_2_15_08_14	Dan River	NC	36.485822	-79.719248	67.50	5.68	1.50	0.43	0.19	1.19	14.89	2.12	22.69
30	Dan_3_15_08_14	Dan River	NC	36.49235	-79.707956	67.43	5.70	1.51	0.44	0.20	1.21	14.88	2.15	22.73
31	Dan_4_15_08_14	Dan River	NC	36.498687	-79.681519	67.32	5.70	1.53	0.44	0.20	1.22	14.95	2.17	22.82
32	Dan_5_15_08_14	Dan River	NC	36.548386	-79.349242	64.21	5.91	1.95	0.60	0.28	1.53	16.49	2.83	25.23
34	Dan_1_16_08_14	Dan River	NC	36.429689	-80.246939	69.80	4.82	0.25	0.04	0.01	0.33	18.69	0.30	23.81
35	CHGA_1_17_08_14	Chattooga River	SC	34.919384	-83.168466	93.44	4.44	0.36	0.16	0.02	0.33	0.44	0.54	5.42
36	FRBR_5_22_08_14	French Broad	TN	35.922139	-82.963111	72.01	10.43	2.29	0.89	0.32	1.81	11.84	3.50	25.77
37	FRBR_4_22_08_14	French Broad	NC	35.877256	-82.772515	68.28	11.90	2.71	1.07	0.38	2.13	13.42	4.16	29.48
38	FRBR_1_23_08_14	French Broad	NC	35.143268	-82.837947	90.46	4.95	0.31	0.03	0.01	0.27	3.53	0.35	8.83
39	FRBR_2_23_08_14	French Broad	NC	35.474275	-82.554778	81.83	8.70	1.19	0.40	0.13	0.97	6.79	1.72	17.21
40	FRBR_3_23_08_14	French Broad	NC	35.569048	-82.563567	68.84	13.61	3.09	1.28	0.47	2.48	10.81	4.84	29.26
41	LTER_1_24_08_14	Little Tennessee	NC	35.233635	-83.393124	79.42	8.30	1.11	0.42	0.11	0.83	7.63	1.64	17.57
42	NOLI_1_22_08_14	Nolichucky	TN	36.125076	-83.181623	59.19	5.31	1.39	0.34	0.13	0.96	31.04	1.86	38.21
43	LTER_4_23_08_14	Little Tennessee	NC	35.354186	-83.503698	81.33	7.30	0.84	0.32	0.08	0.65	7.15	1.24	15.69
44	TELI_1_24_08_14	Telico River	TN	35.396965	-84.276559	91.53	2.44	0.21	0.08	0.02	0.20	3.68	0.31	6.43
45	CHIK_1_01_09_14	Chickamanga	GA	34.708684	-83.658318	85.26	7.51	0.43	0.03	0.00	0.35	2.74	0.46	10.71
46	LCAN_1_07_09_14	Little Cane Creek	SC	34.768476	-83.011242	52.39	10.54	2.41	0.21	0.02	0.94	20.73	2.64	33.91
50	SAMC_1_13_09_14	Sandy Mush Creek	NC	35.684834	-82.779778	76.63	2.63	0.12	0.00	0.00	0.16	18.40	0.12	21.15
51	YADR_1_14_09_14	Yadkin River	NC	36.209268	-80.963598	75.34	4.28	1.07	0.33	0.11	0.86	13.68	1.51	19.47
52	NTOE_1_14_09_14	North Toe River	NC	36.062975	-82.014353	79.05	6.61	0.92	0.41	0.10	0.90	6.69	1.43	14.73
53	CONA_1_20_09_14	Conasauga River	GA	34.95463889	-84.78494444	81.04	2.96	0.38	0.09	0.02	0.31	11.06	0.49	14.51
54	CONA_2_20_09_14	Conasauga River	GA	34.93566667	-84.81305556	79.09	3.11	0.44	0.11	0.03	0.34	12.45	0.58	16.14
55	CONA_3_20_09_14	Conasauga River	GA	34.92891667	-84.83166667	78.71	3.13	0.44	0.11	0.03	0.34	12.77	0.58	16.48
56	CONA_1_21_09_14	Conasauga River	GA	34.90480556	-84.82861111	74.74	3.24	0.43	0.11	0.04	0.34	15.20	0.58	19.02
57	CONA_2_21_09_14	Conasauga River	GA	34.8975	-84.83075	74.44	3.28	0.43	0.11	0.04	0.35	15.29	0.58	19.15
58	CONA_3_21_09_14	Conasauga River	GA	34.91980556	-84.84216667	75.00	3.20	0.43	0.11	0.04	0.34	15.19	0.58	18.97
59	CONA_1_22_09_14	Conasauga River	GA	34.92140351	-84.84163215	75.00	3.20	0.43	0.11	0.04	0.34	15.19	0.58	18.97
60	CONA_2_22_09_14	Conasauga River	GA	34.86147222	-84.83766667	73.04	3.82	0.53	0.12	0.04	0.42	15.99	0.69	20.50
61	GREN_1_28_09_14	Green River	NC	35.278792	-82.343808	77.97	8.94	1.26	0.18	0.02	0.79	8.41	1.46	18.81

62	GREN_2_28_09_14	Green River	NC	35.278189	-82.342103	77.97	8.94	1.26	0.18	0.02	0.79	8.41	1.46	18.81
63	ETOH_1_10_10_14	Etowah River	GA	34.33672222	-84.24544444	82.41	5.93	0.90	0.23	0.07	0.66	5.45	1.20	12.58
64	ETOH_2_10_10_14	Etowah River	GA	34.30041667	-84.27233333	76.63	7.73	1.86	0.39	0.12	1.14	8.05	2.37	18.15
68	HILL.1.6.8.15	Beards Brook	NH	43.111318	-71.918306	87.41	2.94	0.81	0.11	0.00	0.48	2.31	0.92	6.17
71	NEW.1.16.8.15	New River	VA	37.317958	-80.678882	61.56	5.15	1.26	0.50	0.18	1.07	28.78	1.94	35.87
72	PENA 1.6.8.15	Penacook River	NH	43.287155	-71.598952	84.53	3.83	1.58	0.51	0.07	1.09	3.78	2.16	9.77
73	POTR 1.28.7.15	Pootatuck River		41.421329	-73.283369	63.79	12.28	7.99	3.14	0.87	5.87	9.01	12.00	33.29
77	UID 11727	Chicopee River	MA	42.160308	-72.581737	74.49	5.78	2.48	1.34	0.41	2.21	6.25	4.23	16.26
80	UID 11840	Androscoggin River	ME	44.006891	-70.096549	82.39	1.98	1.07	0.42	0.16	0.91	2.38	1.65	6.01
94	UID 14685	Greenbrier River	WV	37.727601	-80.597274	82.97	3.60	0.73	0.20	0.06	0.56	11.03	0.99	15.62
95	UID 14965	Farmington River	CT	41.845109	-72.638406	74.05	8.56	4.77	2.29	0.57	3.95	4.90	7.63	21.09
96	UID 14968 1.28.7.15	Farmington River	CT	41.86917	-72.960619	85.40	5.27	1.45	0.53	0.11	1.07	2.35	2.09	9.71
97	UID 15341	Connecticut River	CT	42.578515	-72.57135	82.86	3.18	1.40	0.59	0.12	2.92	5.93	2.11	11.22
98	UID 15351	Connecticut River	CT	42.602716	-72.570984	82.58	3.17	1.40	0.59	0.12	3.09	5.94	2.11	11.22
100	UID 1728	Connecticut River	CT	42.213946	-72.598955	82.15	3.36	1.56	0.67	0.14	2.95	6.26	2.37	11.99
102	WEST.1.9.8.15	West River	VT	43.022259	-72.656775	90.11	3.14	0.94	0.19	0.03	0.54	3.68	1.16	7.98
103	ETOH_1_17_9_15	Etowah River	GA	34.32086	-84.31797	76.33	7.75	1.81	0.37	0.11	1.12	8.21	2.29	18.25
104	ETOH_2_16.9.15	Etowah River	GA	34.30041667	-84.27233333	76.63	7.73	1.86	0.39	0.12	1.14	8.05	2.37	18.15
105	ETOH_1_14.9.15	Etowah River	GA	34.36168	-84.18673	81.88	6.02	1.20	0.31	0.10	0.78	5.44	1.61	13.07
106	ETOH_1_15.9.15	Etowah River	GA	34.35351	-84.21	82.97	5.69	0.89	0.24	0.07	0.65	5.35	1.20	12.24
107	ETOH_1_16_9_15	Etowah River	GA	34.33672222	-84.24544444	82.41	5.93	0.90	0.23	0.07	0.66	5.45	1.20	12.58
108	MIDO_1_11_4_15	Middle Oconee River	GA	33.96061	-83.44076	43.54	13.19	9.47	2.93	0.79	4.88	21.17	13.19	47.55
111	TUCK_1_14_06_14	Tuckaseegee River	NC	35.36621	-83.25661	86.41	5.78	0.72	0.27	0.06	0.54	3.63	1.05	10.46
112	MIDO_1_12_10_16	Middle Oconee River	GA	33.95684	-83.4377	43.45	13.33	9.55	2.97	0.80	4.95	21.02	13.32	47.67
115	Bluffy	Bluffy Creek	GA	33.893128	-84.924564	80.47	2.63	1.60	1.23	0.70	0.31	0.82	3.53	6.98

117	CHAT_1_16_10_16	Chattooga River	GA	34.79256	-83.32068	93.07	3.63	0.20	0.06	0.01	0.21	1.82	0.27	5.72
118	CHAT_2_16_10_16	Chattooga River	GA	34.78773	-83.32418	90.22	5.17	0.51	0.22	0.05	0.48	2.39	0.78	8.34