QUANTITATIVE ASSESSMENT OF DRIVERS OF ECOSYSTEM FUNCTIONS IN HEADWATER STREAM NETWORKS

by

KAITLIN JEAN FARRELL

(Under the Direction of Amy D. Rosemond)

ABSTRACT

Effectively valuing the contribution of small headwater streams to the functioning of river networks requires an understanding of how physical and biological drivers are linked to ecosystem functions, and of how drivers and functions vary, both within and among stream networks. Despite a large body of work on individual 1st and 2nd order streams, fewer studies have examined patterns in structure or function across different sized streams within a network. Here, I combined field experiments and cross-biome syntheses to link stream structure and function through two focal lenses; the quantity and quality of basal resources, and the role of stream consumers. When developing quantitative relationships between drivers and rates of ecosystem respiration (ER), gross primary production (GPP), and ammonium (NH_4) uptake, I found that within a headwater stream network, coarse and fine benthic organic matter standing stocks were positively correlated with ER, while light availability was a strong driver of GPP. Based on extrapolation to the stream network, small 1st and 2nd order streams are expected to play a substantial role in ER and NH_4 uptake compared to larger streams. Across four distinct stream networks, benthic organic matter carbon to nitrogen and phosphorus ratios were lower in fine than course fractions. This higher relative nutrient content in fine benthic organic matter

suggested that macroinvertebrates feeding on coarse and fine organic matter could be broadly macronutrient or carbon limited, respectively, with implications for in-stream resource cycling. Stream consumers had limited detectable effects on measured metrics of structure and function, both within and among headwater networks. Larval salamanders did not initiate top-down trophic cascades, but reduced stream NH_4 uptake rates, potentially through nitrogen excretion. We also did not detect significant consumer effects across stream networks from five biomes, despite differences in consumer biomass and trophic position. However, across biomes, basal resources were strong drivers of both ER and GPP, which indicated that study networks were bottom-up, rather than top-down controlled. Collectively, these four studies highlight the importance of linking measurements of structure and function in headwater streams, to quantify their role in larger stream networks, and as such, advocate for their protection.

INDEX WORDS: Ecosystem respiration, Gross primary production, Ammonium uptake, Benthic organic matter, Consumer, Macroinvertebrate, Ecological stoichiometry, Coweeta, Cross-biome synthesis

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DEDICATION

To my parents, John and Maggie.

Thank you for giving me roots and wings.

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CHAPTER 1

INTRODUCTION

A river's basic shape… is not a line, but a tree. A river is, in essence, a thing that branches.

- *Porter and Gleick* (1990)

Aquatic ecosystems can be described in terms of their structure, using point measurements of physical, chemical, and biological attributes, and their functions, which integrate multiple in-stream processes by measuring biogeochemical rates in the system (Palmer and Febria 2012). While measures of either structure or function can provide insights into stream condition, linking these two types of measurements is essential to fully understand the health of aquatic ecosystems (Palmer and Febria 2012).

The branching, dendritic structure of stream networks must also be considered in order to fully understand the role of streams in the production, processing, and transport of carbon and nutrients (Cole et al. 2007). In particular, understanding differences in the function of different sized streams within single networks is crucial for the preservation of stream function at the landscape scale (Fisher 1997, Meyer and Wallace 2001). Headwater (1^{st} and 2^{nd} order) streams form the uppermost branches of these river networks, and play an important role in maintaining stream biodiversity (Meyer et al. 2007) and water quality (Lowe and Likens 2005), and provide important linkages to the terrestrial landscape (Baxter et al. 2005). However, these small streams are also among the most at risk, due to degradation of habitat and connectivity (Hawkins 2015). Nutrient pollution is a pervasive threat to headwater streams, with implications for carbon and nutrient processing in the network (Rosemond et al. 2015), while the protection of headwater

stream connectivity is tenuous due to ongoing challenges to federal regulations that protect small streams (Palmer et al. 2010, Hawkins 2015). Thus, while there has been substantial progress in thinking more holistically about streams as interconnected networks or 'riverscapes' (*sensu* Fausch et al. 2002) rather than isolated reaches, a more quantitative understanding of the role of headwater streams in the network is needed to effectively value, and thus protect, them.

Within headwater streams, there is substantial interest in understanding the degree to which ecosystems are structured by top-down (predation) or bottom-up (resource availability) mechanisms. Resource availability, in terms of basal resource standing stocks, is known to affect the productivity of stream ecosystems, in terms of food chain length (Post 2002) and carbon and nutrient cycling (Wallace et al. 1997, 1999, Webster et al. 2000). In many headwater streams, terrestrial detritus provides the main energy source for consumers, and experimental manipulations of detrital standing stocks, through exclusion (Wallace et al. 1997) or accelerated breakdown under nutrient enrichment (Benstead et al. 2009, Rosemond et al. 2015), indicate that bottom-up control limits the production and growth of both macroinvertebrates (Wallace et al. 1999, Cross et al. 2006) and higher consumers (Johnson and Wallace 2005).

In addition to detrital quantity, the quality of basal resources plays a role in carbon and nutrient cycling through freshwater food webs. Ecological stoichiometry, which quantifies the relative carbon and macroelement (e.g., nitrogen [N], phosphorus [P]) content of ecosystem resources (Sterner and Elser 2002), has been identified as a key driver of ecosystem rates, including nutrient uptake (Dodds et al. 2004). Detrital stoichiometry also affects ecosystem rates via the growth and nutrient use efficiency of aquatic bacteria, fungi, and macroinvertebrate consumers (Elser and Urabe 1999, Frost et al. 2005, Cebrian et al. 2009, Hladyz et al. 2009, Manning et al. 2015). Consumer nutrient demands and resource quality can be compared by

estimating threshold elemental ratios (TERs; Sterner and Elser 2002), which estimate the carbon to nutrient ratio at which a consumer changes from carbon to nutrient limitation (Frost et al. 2006). Relating detrital quality to consumer TERs can in turn provide insights into consumer-mediated carbon and nutrient cycling in headwater streams (Elser and Urabe 1999, Tant et al. 2013). Because of its potential to link basal resources and consumers, understanding the natural variability of the elemental content and ratios of organic matter will facilitate predictions about how changes in detrital resources, driven by climate changes or nutrient pollution (Kominoski and Rosemond 2012), could alter ecosystem processes through changes in the efficiency of stream food webs.

The role of consumers in streams, via herbivory and predation that reduce standing stocks of stream primary producers and macroinvertebrates, as well as consumer-driven nutrient cycling, has often been assessed by the experimental reduction or removal of macroconsumers, and the quantification of ensuing changes in the standing stocks of their food resources. Previous research has resulted in mixed results as to the importance of top-down control in shaping ecosystem structure and function (Wooster 1994), and the degree of top-down control appears to depend on a combination of macroconsumer feeding mode (Flecker 1984, Dahl and Greenberg 1996, Cheever and Simon 2009), habitat complexity (Dahl and Greenberg 1996), experimental timing (Cheever and Simon 2009), and the presence of other invertebrate or vertebrate consumers (Dahl and Greenberg 1996, Gibson et al. 2004). To date, the strongest support for top-down control has come from systems where macroconsumer areal biomass is high (Power et al. 1985, Taylor et al. 2006, McIntyre et al. 2008, Capps and Flecker 2013), consumers are primary consumers (Strong 1992, Capps and Flecker 2015), and/or consumer body stoichiometry alters stream nutrient cycling (Small et al. 2009, 2011).

The relative contribution of top-down mechanisms to stream biogeochemical rates is important given the ongoing threats to top consumers in aquatic ecosystems. Human-induced changes to freshwater ecosystems, through altered flow and thermal regimes, nutrient loading, and habitat degradation are altering the species composition of stream communities (Allan 2004, Dudgeon et al. 2006, Woodward et al. 2010). Large aquatic consumers, including fishes, crayfish, and amphibians, are particularly vulnerable to human-induced extirpation, with extinction rates five times higher than for terrestrial species (Ricciardi and Rasmussen 1999). Despite the risk of widespread freshwater community shifts in the face of ongoing climate change (Perkins et al. 2010, Woodward et al. 2010), we do not fully understand the direct and indirect effects of large consumers on aquatic ecosystem processes. Approaches that examine the role of both top-down and bottom-up controls of headwater streams across environmental gradients could help resolve the role of macroconsumers on ecosystem processes, including stream metabolism and nutrient cycling.

Project overview

This dissertation used field experiments and synoptic field collections to address questions of how structure and function are linked within and across headwater stream networks. The research presented was part of the MacroSystems Biology "Scale, Consumers, And Lotic Ecosystem Rates" (SCALER) project, in which researchers assessed how measurements of ecosystem structure and function made at small spatial scales can be used to predict ecosystem processes at the larger scales of the stream reach and river network. In addition, collaborators in this project sought to examine the degree to which top-down control by aquatic macroconsumers controlled ecosystem rates. We conducted the SCALER project in five headwater stream

networks from distinct biomes across North America, including tropical rainforest, temperate deciduous forest, tallgrass prairie, boreal forest, and Arctic tundra. My dissertation combines field experiments conducted at the United States Department of Agriculture Forest Service Coweeta Hydrologic Laboratory, a Long Term Ecological Research (LTER) site in the Southern Appalachian Mountains of Macon County, North Carolina, USA, with cross-biome syntheses from streams across the SCALER project to address questions about the role of basal resources in driving ecosystem functions, and the importance of top-down control in the structure and function of headwater streams.

Chapter 2: Spatial and temporal patterns in drivers of ecosystem functions in a headwater stream network

The objectives of this study were to isolate drivers of ecosystem respiration (ER), gross primary production (GPP) and ammonium (NH_4) uptake across a headwater stream network to determine how rates change across small environmental gradients. Specifically, I used recirculating chambers to measure ER, GPP, and NH_4 uptake in 9 streams across two seasons and link rates to physical (temperature, light) and biological (benthic organic matter, chlorophyll-a) drivers. I then used reach-scale water temperature and light data to scale-up chamber rates to estimate how each function changed across a headwater stream network. Finally, I used delineations of the extent of headwater streams in the network to estimate the relative contribution of 1^{st} and 2^{nd} order streams to ecosystem processes within 1^{st} – 4^{th} order streams in the network. This approach improves our understanding of the variability in ecosystem functions across a headwater stream network by developing quantitative relationships between drivers and rates, and emphasizes the role headwater streams play in network-scale functions.

Chapter 3: Detrital resource stoichiometric C:N:P baselines in stream ecosystems

In this study, I characterized the stoichiometry of two detrital resource pools, coarse (CBOM) and fine (FBOM) benthic organic matter, across four stream networks within the SCALER project. I sought to determine whether nutrient content (carbon [C], nitrogen [N], and phosphorus [P]) and stoichiometric ratios (e.g., C:N, C:P) differed between CBOM and FBOM, both within a single stream network and across diverse networks. I predicted that FBOM N and P content would be consistently higher, and thus C:N and C:P ratios lower, across sampled networks due to the high surface area for microbial colonization of FBOM compared to CBOM. I also compared the CBOM and FBOM stoichiometry in each stream network to estimated TERs of macroinvertebrate shredders and collectors, which feed on CBOM and FBOM, respectively. This assessment allowed for a first estimation of the potential for carbon and nutrient (N and/or P) limitation in the macroinvertebrates, based on consumer-resource mismatches, which has implications for stream carbon and nutrient cycling. By identifying differences in the stoichiometry of two types of basal resources within and among networks, we can better understand the broad patterns of resource quality and the potential for carbon and nutrient limitation by stream detritivores.

Chapter 4: Effects of altered larval salamander biomass on ecosystem structure and function in a headwater stream

In this study, I tested the role of a top consumer in fishless headwater streams to assess the extent of top-down versus bottom-up control of ecosystem functions. Specifically, I used an enclosure experiment to examine whether larval salamander biomass reduced leaf litter

breakdown rates, ecosystem respiration, and ammonium uptake in a headwater stream in Coweeta via predation on shredding macroinvertebrates. While the southeastern United States is a current hotspot of salamander species richness (Petranka 1998), ongoing pressures from land use and climate changes may substantially alter habitat suitability for many species whose larvae inhabit fishless headwater streams (Peterman et al. 2008, Milanovich et al. 2010). This threat underscores the importance of understanding how changes in salamander biomass affect headwater streams, so that we can anticipate ecosystem-level changes in stream structure and function if salamander populations decline.

Chapter 5: Comparing effects of aquatic macroconsumers in diverse headwater stream networks

In this study, I synthesized results of experimental short-term consumer exclusions across the five SCALER networks, with the goal of determining whether we could detect cross-site patterns in top-down (consumer) or bottom-up (resource) control of ecosystem functions (ER, GPP, NH_4 uptake). The consistent experimental design across sites allowed us to compare the effects of consumer trophic group and density on a common suite of basal resource standing stocks and ecosystem rates, despite differences in the identity, diversity, and abundance of macroconsumers both within and among stream networks. Specifically, I identified basal resource drivers of ER, GPP, and NH_4 uptake, and quantified top-down macroconsumer effects on drivers. This approach provides a unique cross-site perspective of the importance of top-down controls of ecosystem structure and function.

Together, the chapters of this dissertation demonstrate the importance of understanding top-down and bottom-up effects of consumers and basal resources in driving ecosystem rates within and among headwater streams. In addition, this work underscores the importance of using

a network approach to understand stream structure and function, and the value of cross-biome synthesis to better understand the generality of observations made in single streams.

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CHAPTER 2

SPATIAL AND TEMPORAL PATTERNS IN DRIVERS OF ECOSYSTEM FUNCTIONS IN A HEADWATER STREAM NETWORK[1]

[1] Farrell, K.J., A.D. Rosemond, J. Rüegg, F. Ballantyne IV, and J.S. Kominoski. To be submitted to *Freshwater Science*.

Abstract

Network position has increasingly been shown to define unique characteristics and roles of streams. Here, we quantified ecosystem functions (ecosystem respiration [ER], gross primary production [GPP], and ammonium [NH_4] uptake) in 1^{st} – 4^{th} order streams using recirculating chambers to isolate predicted physical (temperature, light) and basal resource (coarse [CBOM] and fine [FBOM] benthic organic matter, chlorophyll-a) drivers of rates and test how they varied across space in a headwater network. Drivers differed more between two seasons than across the sampled network; specifically, higher light in the spring (April) lead to increased GPP, and higher standing stocks of CBOM and FBOM in the fall (September) led to increased chamber ER. Ammonium uptake was marginally related to chamber ER, but CBOM and FBOM had limited explanatory power. We also used reach-scale temperature and light data to scale up our chamber estimates to areal rates representative of stream reaches, which we used to assess changes in reach-scale ER, GPP, and NH_4 uptake as a function of network position. Estimated reach-scale ER and NH_4 uptake did not change with distance downstream or aspect, though scaled GPP increased with distance downstream in September due to increased light availability. Finally, we combined our scaled rates with published relationships between stream order and wetted area to make a conservative estimate of the contribution of 1^{st} and 2^{nd} order streams to the functions of our 1^{st} – 4^{th} order network, which indicated that the smallest streams play a sizeable role in ER (76%) and NH_4 uptake (69%), and also contribute substantially to network GPP (50%). Together, these findings suggest that chambers can be useful for isolating drivers of ecosystem rates, and that consideration of seasonal and spatial variability is key to estimating of the role of different sized streams in headwater networks.

Introduction

Headwater (1st and 2nd order) streams form the uppermost branches of river networks, and their small drainage areas and limited cumulative upstream effects result in substantial ecological and hydrological heterogeneity relative to larger streams (Lowe and Likens 2005). These small streams are essential for maintaining freshwater biodiversity (Meyer et al. 2007) and water quality (Lowe and Likens 2005), and for the exchange of materials with surrounding terrestrial landscapes (Baxter et al. 2005). Because of their small catchments and high biodiversity, headwater streams are considered particularly sensitive to disturbance and degradation (Meyer et al. 2007). Headwater streams are also abundant in the landscape, and likely account for over 73% of stream channel length in the United States, based on recent improvements in mapping of perennial headwater streams (Leopold et al. 1964, Benstead and Leigh 2012).

Small streams form an essential link in connecting terrestrial landscapes to larger rivers, and ultimately, the ocean. Streams play an active role in the transformation and storage of terrestrial carbon transported along the land-ocean aquatic continuum, as well as the production of carbon *in situ* (Cole et al. 2007). Quantifying how headwater and larger streams transport and transform materials is an important step in effectively valuing these ecosystems from a management perspective. Estimates of stream gross primary production (GPP), ecosystem respiration (ER), and nutrient uptake rates provide a snapshot of processes that integrate multiple in-stream processes (Palmer and Febria 2012), and help us understand the contribution of headwater streams to the functioning of larger river networks. Understanding how the structural drivers and functional rates differ throughout stream networks is in turn key to predicting how lotic ecosystems may respond to future changes in climate, land use, and water extraction.

It is unclear to what extent biogeochemical processes vary among headwater streams within a network. Within the River Continuum Concept (RCC), Vannote et al. (1980) predicted that there are rapid changes in rates of ecosystem functions from 1^{st} – 4^{th} order streams, as allochthonous organic carbon decreases and autochthonous production increases with stream size. These qualitative predictions have been supported by cross-site studies, which found that GPP and the ratio of carbon production to respiration (P:R ratio) increased with light availability and watershed area (Mulholland et al. 2001, Finlay 2011), and that nitrogen uptake length increased in larger streams (Webster et al. 2003). However, we understand less about finer-scale, quantitative differences in the drivers and rates of metabolism and nutrient uptake within headwater stream networks, and whether 1^{st} and 2^{nd} order streams function differently than larger 3^{rd} and 4^{th} order streams. In order to better understand how streams of different sizes function within a network, measurements from streams along a size gradient within single catchments, rather than from single sites across multiple catchments, are needed (Fisher 1997, Meyer and Wallace 2001).

Whole-stream measurements integrate multiple interacting processes in streams, but patch-scale measurements can allow researchers to assess mechanistic drivers of ecosystem processes by isolating specific variables of interest from other confounding environmental factors (Rüegg et al. 2015). Recirculating chambers have been used to compare habitat-specific rates of stream metabolism (e.g., Uzarski et al. 2001) and nitrogen uptake (e.g., O'Brien and Dodds 2008), as well as to quantify inter-biome differences in metabolism along a river continuum (Bott et al. 1985). Recirculating chambers can also be used to assess isolated drivers of metabolism and uptake across gradients of stream size and between seasons within headwater networks by controlling for the effects of discharge and gas exchange on rate estimates.

Our objectives in this study were three-fold: (1) to quantify rates of GPP, ER, and ammonium (NH_4) uptake in 1^{st} – 4^{th} order streams in a headwater network in 2 distinct seasons, (2) to identify drivers of ecosystem rates across stream reaches in the network and (3) to test whether estimated whole-stream rates, when scaled from chamber rates to reflect stream-level light and temperature regimes, changed in a predictable way across a headwater stream network. To achieve these objectives, we used recirculating chambers to estimate patch-scale GPP, ER, and NH_4 uptake in streams throughout the Coweeta Creek basin during spring (April) and early fall (September). We assessed the importance of different physical and biological drivers on ecosystem rates during each season and in each stream. We then scaled chamber rates to incorporate light and temperature regimes from the whole-stream scale, and used the scaled rates to assess whether reach-scale ER, GPP, and NH_4 uptake change as a function of network position. Finally, we used previously published network estimates of the benthic area of 1^{st} – 4^{th} order streams to estimate the contribution of 1^{st} and 2^{nd} order streams to the overall function of the headwater network.

Within a season, we did not expect all small streams to behave similarly; that is, we predicted that our chambers would detect differences in rates of metabolism and uptake between 1^{st} and 2^{nd} order headwater and larger 3^{rd} and 4^{th} order streams. We predicted that GPP would be strongly influenced by light availability and chlorophyll-a, and that these 2 factors, and thus GPP, would increase with distance downstream. In contrast, we predicted that detrital carbon standing stocks would be a significant predictor of ER and NH_4 uptake, due to strong associations between heterotrophic microbes and detrital carbon, and that ER would be highest in small, retentive 1^{st} order sites where benthic organic matter standing stocks are highest (Webster 2007). Finally, we predicted that scaled chamber rates would indicate higher net heterotrophy

(lower P:R) in headwater streams, which would decrease downstream (higher P:R) as canopy cover decreased and irradiance increased across the $1^{st} - 4^{th}$ order stream network (Hagen et al. 2010).

Methods

Site description

We conducted our study in a total of 9 streams within the US Forest Service Coweeta Hydrologic Laboratory in Macon County, North Carolina, USA that encompassed a gradient of discharge, elevation, and upstream drainage area (Table 2.1, Fig. 2.1). Field measurements were conducted in April 2013 (3 streams) and September 2014 (6 new streams; 9 streams total). Because we had measurements from 3× as many streams in September, our design, and thus analyses, were unbalanced with regard to the extent of the stream network sampled during each season and the number of replicate samples per stream each season.

For each stream, an experimental reach was delineated, with reach length assigned proportional to stream size. Reach length ranged from 50 m in our smallest headwater sites to 120 m in the larger 4^{th} order streams (Table 2.1). Reach-scale temperature and light were monitored at the top and bottom of the reach using a YSI ProODO sensor (Yellow Springs, Ohio, USA) and an Odyssey photosynthetically active radiation (PAR) recorder (Dataflow Systems Limited, Christchurch, New Zealand). Both light and temperature were logged at 10-minute intervals during the experimental period.

We measured physical and biological characteristics of our streams during each experimental period. Stream discharge at each site was quantified by releasing a slug of sodium chloride (NaCl) and monitoring specific conductance at the downstream station of the reach

(Gore 2006). Discharge was quantified at least 3 times per stream reach during each experimental period. Streambed elevation, upstream drainage area, and aspect were calculated from a 60 × 60 m digital elevation model of the Coweeta Creek basin, and distance downstream was calculated based on a 10 × 10 m raster layer of the stream network (Rosemond et al. 2015).

Rate measurements and sample processing

Recirculating 10 L (0.01 m^3) chambers were used to quantify patch-scale metabolism (ER, GPP) and NH_4 uptake (see Rüegg et al. 2015 for detailed methods). Briefly, plastic mesh baskets (0.01 m^2 each) were filled with representative mixed gravels and cobbles from each study reach and installed in the streams in groups of 5 baskets. Basket groups were installed haphazardly in riffle and run habitats and embedded flush with surrounding substrates. In 2013, 8 replicate groups of baskets were placed in each stream (n = 3 streams), and allowed to incubate for 31-32 days. In 2014, 5 (n = 6 streams) or 10 (n = 3 streams) replicate groups of 5 baskets were placed in each stream, and allowed to incubate for 26-30 days.

After incubation, sets of 3 baskets from each group were placed in a chamber, with 6 (2013) or 5 (2014) chambers run simultaneously on the streambank immediately adjacent to each study reach. For chamber metabolism measurements, each chamber was fitted with a YSI ProODO sensor and an Odyssey PAR recorder, which both logged at 1-minute intervals. Ecosystem respiration was quantified in covered chambers for 15 (2013) or 30 minutes (2014), followed by an additional 15 (2013) or 30 minutes (2014) of net ecosystem production (NEP) in ambient light conditions. Following NEP measurements, DO and light loggers were removed. A 3 mL slug of 0.46 g L^{-1} NH_4Cl stock solution was then added to each chamber to achieve a target chamber-water concentration of 50 μg L^{-1} NH_4-N. Water samples were withdrawn 1, 3, 6, 12, 24,

and 40- minutes post-slug addition to quantify NH_4 uptake in each chamber. Water samples were immediately filtered through a 0.7 μm glass fiber filter (GF/F, Sterlitech Corporation, Kent, Washington, USA), cooled on ice, and frozen upon return to the laboratory. Chambers were thoroughly rinsed with stream water between runs.

In the laboratory, frozen samples were defrosted and immediately analyzed for NH_4-N using the colorimetric phenol-hypochlorite method (APHA 1995) and read on a spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) at 640 μm wavelength.

Organic matter collection and processing

Organic matter (coarse benthic organic matter [CBOM], fine benthic organic matter [FBOM], algal chlorophyll-a [chl-a]) was collected for each group of baskets using the 2 baskets not run in chambers. One of the baskets was used to collect chl-a and FBOM; the basket was removed from the stream and quickly placed into a bucket with 5 L of stream water. Surface substrates were removed from the basket and placed in a polyethylene bag for chl-a analysis. Remaining contents of the basket were dumped into the water, thoroughly agitated, and then a 250 mL subsample was collected to quantify FBOM. Coarse benthic organic matter was quantified from the other remaining basket. For CBOM, the basket was removed from the stream, immediately placed in a 250 μm sieve, and transferred to an enamel pan. Substrates were rinsed with stream water and removed, and remaining CBOM was rinsed into polyethylene bags and preserved in 8% formalin.

In the lab, the FBOM slurry was resuspended and filtered through a pre-weighed 0.7 μm GF/F until it clogged. Filtered volume was recorded and filters were dried at 50°C for at least 72

hours, then weighed for dry mass. Samples were then ashed at 500°C for 4.5 hours and reweighed for ash-free dry mass (AFDM). Algal biomass was estimated as the chl-a concentration on collected substrates. Chlorophyll-a was extracted from collected cobbles and gravels using hot ethanol (Sartory and Grobbelaar 1984) and concentrations were quantified using a spectrophotometer (Shimadzu UV-1800) with an acidification correction for phaeophytin (APHA 2005, Parker et al. 2016). In 2013, we conducted whole-substrate hot ethanol extractions. In 2014, we scrubbed each substrate using a hard-bristled plastic brush, and filtered the slurry of dislodged material through a pre-weighed 0.7 μm GF/F, which was frozen for up to 2 weeks. Chlorophyll-a was then extracted from filters using hot ethanol. Chlorophyll-a was extrapolated to a per-area basis using the surface area of scrubbed substrates, as estimated from volume displacement-surface area relationships (K. Farrell, unpublished data). Processing of CBOM was conducted by the Whiles lab at Southern Illinois University; CBOM was manually separated from macroinvertebrates using a dissecting microscope, then dried, weighed, and ashed as described above for FBOM.

Estimating chamber GPP, ER, and NH_4 uptake rates

We calculated chamber metabolism rates (ER_{cham}, GPP_{cham}) based on the linear change in dissolved oxygen concentration over time: $\frac{d[O_2]}{dt} = \left(\frac{\Delta O_2}{t}\right)\left(\frac{V}{A}\right)$, where ΔO_2 is the change in dissolved oxygen concentration in the chamber (mg L^{-1}), t is the elapsed time during the ER or NEP measurement (min), V is the chamber volume (L), and A is the area of the chamber substrates in the set of 3 baskets (0.03 m^2). Separate calculations were made for ER and NEP, based on the start and end times of the dark and ambient light periods, respectively. GPP_{cham} was

then calculated as $GPP_{cham} = NEP_{cham} + |ER_{cham}|$, where ER_{cham} is always < 0. P:R ratios were calculated for each chamber run by dividing GPP_{cham} by ER_{cham}.

Chamber NH_4 uptake was calculated as the linear change in NH_4 concentration in sampled chamber water over time: $U_{cham} = \left(\frac{\Delta NH_4}{t}\right)\left(\frac{V}{A}\right)$, where ΔNH_4 is the change in chamber NH_4 concentration (μg L^{-1}), t is the elapsed time (min), V is the chamber volume (L), and A is the area of the chamber substrates (0.03 m^2).

Scaling chamber rates

To assess whether chamber ER and GPP rates changed predictably across a stream network, we first corrected chamber ER rates to the water temperatures in the stream reach using: $ER_{reach} = \frac{10(ER_{cham})}{1.045^{T_{cham}-T_{reach}}}$, where T_{cham} and T_{reach} represent the water temperatures at each logging point in the chamber and reach, respectively, and 1.045 is the Arrhenius coefficient for the effect of temperature on respiration (Parkhill and Gulliver 1999). Chamber rates were multiplied by 10 to account for differences in the logging interval between chamber (1 minute) and whole-reach (10 minute) data. We calculated 144 estimates of ER_{reach} per day (6 points per hour × 24 hours), which were summed and converted from milligrams to grams to estimate daily whole-stream ER (g m^{-2} d^{-1}) based on chamber rates.

To scale GPP_{cham}, we first estimated chamber parameters of P_{max} (rate of photosynthesis at PAR saturation) and α (slope of the relationship between photosynthesis and PAR when light is low; Jassby and Platt 1976) by fitting GPP_{cham} rates to mean PAR during each chamber run using the nonlinear least squares (nls) function in the R stats package. We used the estimated P_{max} (≈ 0.963) and α (≈ 0.019) to estimate reach-scale GPP (GPP_{reach}) based on differences in

chamber- and reach-scale light and temperature conditions using: $GPP_{reach} = \frac{P_{max}\tanh\left(\frac{\alpha PAR_{reach}}{P_{max}}\right)}{1.036^{T_{cham}-T_{reach}}}$, where PAR_{reach} is the light intensity logged in the stream reach and 1.036 is the Arrhenius coefficient for the effect of temperature on photosynthesis (Parkhill and Gulliver 1999). As with the scaled ER, 144 estimates were summed and converted from milligrams to grams to estimate daily whole-stream GPP (g m^{-2} d^{-1}).

Scaled ER and GPP were compared to previously published whole-stream rates from Coweeta streams (Mulholland et al. 1999, Bernot et al. 2010, Hagen et al. 2010, Northington et al. 2013) to assess whether scaled rates over- or under-estimated whole-stream ER and GPP. We used Pearson correlation to assess the relatedness of scaled ER and GPP for both our data alone, and when including our data with literature values of whole-stream rates.

To estimate the contribution of 1st and 2nd order streams to network-scale metabolism, we combined our scaled rates of GPP, ER, and NH_4 uptake with previously published estimates of streambed area in 1st – 4th order streams throughout the Little Tennessee River network that includes the Coweeta Creek drainage (Rosemond et al. 2015). We then calculated the proportion of the total function (e.g., GPP, ER, NH_4 uptake) performed by 1st and 2nd order streams in the 1st – 4th order portion of the network. For this analysis, we estimated areal GPP and ER in grams of carbon using standard calculations based on respiratory and photosynthetic quotients (Bott 2006).

Statistical analyses

We tested whether hypothesized drivers changed across the network and between seasons using linear mixed effects models (lme4 package; Bates et al. 2014). To test whether

hypothesized drivers were significant predictors of chamber ER or GPP, we used models with a nested random effect of stream within season to account for non-independence of replicate chamber runs (n = 5 – 10) within a stream and unquantified differences between sampling seasons. Hypothesized drivers for chamber ER included standing stocks of CBOM and FBOM and chamber water temperature, and hypothesized drivers for chamber GPP included chl-a and PAR. Chamber water temperature was not included in the GPP models as it has been shown to be strongly correlated with light (Valett et al. 2008), which was also the case in our chambers (Pearson correlation: r (80) = -0.71, $p < 0.001$). Ammonium uptake rates were modeled with CBOM, FBOM, chl-a, and PAR as hypothesized drivers. Ammonium samples from April 2013 were compromised during analysis so were not included in model selection. For all models, drivers and chamber rates were natural log transformed to meet assumptions of normality. We developed candidate models that included all potential interactions, and we simplified models using a stepwise approach to remove interactions and replace them with additive effects. The simplest candidate models for each rate did not include any drivers (i.e., ~ intercept only). We used the AICcmodavg package (Mazerolle 2016) to rank candidate models using AIC_c to account for small sample sizes (Burnham and Anderson 2002). Parameter estimates, standard errors, and p-values of drivers were quantified using the lmerTest package (Kuznetsova et al. 2016). Conditional R^2 values were calculated to assess goodness of fit of candidate models (Nakagawa and Schielzeth 2013) using the MuMIn package (Bartoń 2016).

To test whether chamber rates of NH_4 uptake or scaled rates of ER and GPP changed along the stream network, we used mixed effects models with a random effect of stream to test whether rates changed predictably with distance downstream and stream aspect. Upstream drainage area was not included in the network models as it was strongly correlated with distance

downstream (Pearson correlation: r (81) = 0.93, $p < 0.001$). Model selection criteria was the same as described above. Literature values of GPP, ER, and NH_4 uptake from Coweeta streams were used to calculate confidence intervals for whole-stream measurements to assess whether our scaled rate estimates fell within these confidence intervals. All analyses were conducted using R 3.3.2 (R Core Team 2016).

Results

Temperature, light, and BOM across the network

To understand changes in ecosystem rates across the network, we first assessed how predicted rate drivers changed over space and between seasons. Potential drivers of ecosystem rates varied more between seasons than across the network within either season. Both season and elevation affected stream water temperature (season × elevation interaction; $F_{1,22201} = 1469.02$, $p < 0.001$, $R^2_c = 0.82$). During both seasons, water temperature was inversely related to elevation (Appendix A, Fig. 2.2A); in April, the lowest elevation stream was 1.58× warmer than the highest elevation stream ($F_{1,3} = 9.51$, $p = 0.054$, $R^2_c = 0.18$), versus a difference of 1.33× in September ($F_{1,9} = 33.23$, $p < 0.001$, $R^2_c = 0.97$). However, the difference in temperature was greater between seasons than across the network within a season; mean water temperatures were over 2× warmer in September than in April (16.99 ± 0.01 vs. 8.01 ± 0.04°C).

Light availability at the stream surface (PAR) also varied more between seasons than across the network within a season. There was a significant interaction effect between season and distance downstream on PAR (season × distance downstream interaction; $F_{1,13349} = 60.38$, $p < 0.001$, $R^2_c = 0.34$). During both seasons, light increased with distance downstream (Appendix A, Fig. 2.2B); in April, light was 7.8× higher at the most downstream than the most upstream site

($F_{1,3}$ = 8.15, p = 0.065, R^2_c = 0.15), and in September, the difference was 4.6× ($F_{1,9}$ = 13.43, p = 0.005, R^2_c = 0.26). Mean light availability in April, prior to leaf-out, was nearly 8× higher than in early September, when the canopy was closed (Table 2.1, Fig. 2.2B).

Benthic organic matter standing stocks did not significantly change across the stream network, but differed between seasons. Both CBOM and FBOM standing stocks were significantly higher in September than in April (Appendix A, Fig. 2.2C-D). In September, CBOM standing stocks were over 6× higher than in April ($F_{1,24}$ = 73.19, p < 0.001, R^2_c = 0.50), and FBOM standing stocks were over 11× higher than in April ($F_{1,70}$ = 160.50, p < 0.001, R^2_c = 0.76).

Drivers of chamber rates

Chamber ER was driven by both BOM and water temperature, and light was a significant predictor of chamber GPP. Model selection for ER_{cham} yielded 4 models with substantial support ($\Delta AIC_c \leq 2$), all of which included CBOM as a significant driver (Table 2.2, Fig. 2.3A). The second top model for ER_{cham} also included a marginally significant interaction between CBOM and chamber water temperature (Table 2.2), and FBOM was marginally significant in the third model for ER_{cham} (Table 2.2, Fig. 2.3B). When testing the effect of chl-a and PAR on GPP_{cham}, there was only 1 model with substantial support ($\Delta AIC_c \leq 2$), which included a significant effect of PAR (Table 2.2, Fig. 2.3C). Despite having chl-a as a predicted driver (Fig. 2.3D), it was not retained in the top model. For GPP_{cham}, the effect of water temperature was not included in model selection, due to its significant negative correlation with PAR across seasons (Pearson correlation: r (80) = -0.71, p < 0.001). When tested as a separate model, water temperature was a significant predictor of GPP_{cham} (t_{78} = -7.60, p < 0.001, R^2_c = 0.43).

Seasonal differences in drivers led to differences in chamber rate estimates between seasons (Fig. 2.3). In fall, higher CBOM and FBOM standing stocks led to higher ER_{cham} estimates; 1.4× higher in September than in April (Fig. 2.3A-B; $F_{1,79} = 5.58$, $p < 0.02$, $R^2_c = 0.07$). In contrast, during April, when PAR was higher, estimates of GPP_{cham} were 5× higher than in September (Fig. 2.3C; $F_{1,9} = 32.70$, $p < 0.001$, $R^2_c = 0.39$). These rates led to seasonal differences in whether chambers were net autotrophic (P:R > 1) or net heterotrophic (P:R < 1) during the daylight conditions under which they were run (Appendix A). In April, GPP_{cham} often exceeded ER_{cham}, leading to net autotrophic conditions in 74% of chamber runs. In contrast, in September, ER_{cham} exceeded GPP_{cham} in all chambers, resulting in net heterotrophy. However, during both seasons, extrapolation to diel rates (i.e., 24 hours of ER, 12 hours of GPP) resulted in net heterotrophy in chambers.

Our models of basal resource drivers of NH_4 uptake rates did not have substantial explanatory power (Table 2.2). The top model for NH_4 uptake when assessing basal resource drivers included CBOM, FBOM, chl-a, and PAR, though none of the drivers were significant predictors of NH_4 uptake and explanatory power was low (Table 2.2, $R_c^2 = 0.12$). When modeled as a function of rates (ER_{cham} and GPP_{cham}), only ER_{cham} was a marginally significant predictor of NH_4 uptake (Table 2.2, Fig. 2.3E) and had minimal explanatory power ($R_c^2 = 0.10$). Chamber GPP was not related to NH_4 uptake (Table 2.2, Fig. 2.3F). Most estimates of NH_4 uptake exhibited net NH_4 uptake (65.4%), though some runs resulted in estimated net mineralization (5.8%), or non-detectable changes in NH_4 concentration (28.8%).

Scaled GPP, ER, and NH_4 uptake across the headwater network

We tested whether GPP and ER differed across the stream network and between seasons by scaling chamber rates to reach-scale daily rates using diel temperature and light data. The top model for scaled ER indicated rates were significantly higher in September than in April, but that there were no significant changes in ER as a function of distance downstream during either season (Table 2.3, Fig. 2.4A). For GPP, there was a significant interaction between season and distance downstream (Table 2.3, Fig. 2.4B). April GPP was higher on average but did not change predictably as a function of distance downstream, though the extent of the network captured in April was slightly truncated compared to September (April range: 4.04 km; September range: 4.52 km). In September, scaled GPP increased with distance downstream (Fig. 2.4B). Based on model fits, the most downstream site had GPP rates over 5x higher than in the most upstream site (Fig. 2.4B). During both seasons, scaled GPP was very low (~0 – 0.08 g O_2 m^{-2} d^{-1}), and all estimates indicated that streams throughout the network were strongly heterotrophic (P:R < 1, Fig. 2.5). When scaled rates were combined with previous whole-stream studies from Coweeta (Mulholland et al. 1999, Bernot et al. 2010, Hagen et al. 2010, Northington et al. 2013), estimates of daily ER and GPP were significantly positively correlated (Fig. 2.5, Pearson correlation $r(104) = 0.59$, $p < 0.001$). NH_4 uptake rates in September did not significantly change as a function of distance downstream (Table 2.3, Fig. 2.4C). Stream aspect was not retained in the top models for any of the scaled rates.

When extrapolating to the larger Little Tennessee network, 1st and 2nd order streams accounted for 81% of the stream length and 66% of wetted stream area among 1st – 4th order streams (Table 2.4). Functionally, these small streams represented 76% of daily ER, 50% of

daily GPP, and 69% of daily NH_4 uptake among 1^{st} – 4^{th} order streams in the Little Tennessee drainage (Table 2.4).

Discussion

Drivers of metabolism and nitrogen uptake in a headwater stream network

Our chamber measurements identified light and benthic organic matter standing stocks as key drivers of GPP and ER, respectively, that allow whole-stream rates to be estimated between seasons and across the stream network. Light intensity (PAR) was strongly correlated with GPP in chambers across seasons (Fig. 2.3C), and accounted for 35% of the variance in measured GPP (Table 2.2). In April, mean PAR during GPP measurements was over 11× higher than in September, and resulted in spring GPP_{cham} was 5× higher than in fall. Our measured effect of PAR on chamber GPP was consistent with previous studies that found that GPP was positively correlated with PAR in streams across large environmental gradients (Mulholland et al. 2001, Bernot et al. 2010, Finlay 2011), and indicates that chambers can quantify drivers of GPP despite their small size and short run times.

Standing stocks of CBOM and FBOM were correlated with chamber ER across seasons (Fig. 2.3A, B), and CBOM alone explained 15% of the variance in measured ER (Table 2.2). Measured CBOM and FBOM were 8× higher in September than April, and this difference was associated with a 1.4× increase in ER rates in fall than spring, from 0.34 to 0.47 mg O_2 m^{-2} min^{-1}. Our identification of quantitative relationships between benthic organic matter and ER mirrors the role of CBOM and FBOM in driving ER in stream networks across broad environmental gradients (Farrell et al. *in prep*), and reinforces the utility of chambers in isolating drivers of different ecosystem rates within, as well as across, headwater networks.

By using recirculating chambers with consistent water velocities and depths across a network, we were able to examine drivers of NH_4 uptake not related to those two hydrologic variables, which are considered 'primary determinants' of nitrogen uptake (Peterson et al. 2001). We did not detect a change in NH_4 uptake rate due to basal resource standing stocks, though our chambers did identify a weak positive relationship between ER and NH_4 uptake rate, with a 21% increase in NH_4 uptake across the range of measured chamber ER (0.05 – 3.33 mg O_2 m^{-2} min^{-1}). This finding is consistent with previous cross-biome measurements in both whole streams (Webster et al. 2003) and chambers (Fellows et al. 2006) that found positive relationships between ER and NH_4 uptake. Because both our chambers and previous cross-system reach-scale studies (Webster et al. 2003) were unable to identify biological drivers of NH_4 uptake, further investigation is needed to pinpoint the role of basal resource standing stocks in NH_4 uptake throughout headwater networks.

Spatial and seasonal changes in rates across a headwater stream network

Our scaled metabolism rates indicated that for whole-stream GPP and ER in retentive, forested streams, rates differed more between seasons than across the network, but that network position was an important consideration for estimating GPP. Scaled GPP increased with distance downstream, but only during early fall, when our sampling captured larger gradients of light along the stream network and when light is generally limiting for GPP in forested headwater streams (Rosemond 1994). In September, GPP in the most downstream site was 3.7× higher than in the most upstream site. In contrast, across the sampled network, estimated GPP was 5.1× higher in April than in September, due to higher light availability prior to leaf out across the portion of the network we sampled.

Unlike GPP, scaled ER did not significantly change with distance downstream within the sampled headwater network. However, scaled ER was significantly different between seasons, with 1.4× higher rates in September, due to higher temperatures and organic matter standing stocks. Organic matter standing stocks in Coweeta streams are typically near an annual minimum in September, with CBOM standing stocks approximately 10% of the annual maximum (A.D. Rosemond, unpublished data). At the time of our April sampling, CBOM standing stocks are typically higher, at 57% of the annual maximum (A.D. Rosemond, unpublished data). We had therefore anticipated that standing stocks of organic matter, and thus ER, would be higher in April than in September, but our measured standing stocks and ER were higher in September than April. This may be due to substantially higher precipitation (Shepherd et al. 2017) and stream discharge in spring 2013 versus fall 2014 (Table 2.1), which could have flushed organic matter from streams and baskets prior to our spring 2013 measurements.

Our patterns of GPP and ER were consistent with longitudinal studies in the mainstem of the Little Tennessee River, that our stream network drains into, where GPP increased 3-fold over a stretch of 36 km of river length but ER did not significantly change (McTammany et al. 2003). Our findings within this headwater network also aligned with broad predictions from the RCC, in that light availability and thus GPP both increased downstream (Vannote et al. 1980, Lamberti and Steinman 1997). However, contrary to the RCC prediction that a transition from net heterotrophy to net autotrophy would occur around 3rd order streams (Vannote et al. 1980), study sites throughout Coweeta (1st – 4th order) were net heterotrophic. In addition, our scaled rates indicated that patterns of GPP increasing in larger streams detected across large environmental gradients (Lamberti and Steinman 1997) are also present across a much smaller within-network

gradient, and emphasize the importance of developing quantitative relationships that relate drivers and rates to stream size within single networks.

Scaled chamber rates compared to whole-stream measurements

When chamber rates of NH_4 uptake were scaled up to a daily time-step, rates were comparable to previously published whole-stream rates for Coweeta (0.05 – 0.10 g N m^{-2} d^{-1}; Hall et al. 1998; Tank et al. 2000; Webster et al. 2003; Payn et al. 2005; Northington et al. 2013; K. Farrell unpublished data), indicating that our methods are useful for estimating reach-scale nitrogen dynamics. In contrast, estimates of reach-scale ER and GPP were generally low compared to previously published rates for similar-sized streams in Coweeta. Streams throughout Coweeta tend to have low GPP due to heavy riparian shading (mean = 0.08 g O_2 m^{-2} d^{-1}; Mulholland et al. 1999, Bernot et al. 2010, Hagen et al. 2010, Northington et al. 2013), but previously published estimates were 2× higher than our mean scaled GPP (Fig. 2.5). Similarly, the mean rate of ER from previous studies (3.42 g O_2 m^{-2} d^{-1}) was over 5× higher than our mean scaled ER (0.59 g O_2 m^{-2} d^{-1}; Fig. 2.5). However, direct comparisons of our scaled rates to previous studies should be interpreted with some caution, because whole stream methods can overestimate ER due to both errors in reaeration in small streams (Webster 2007) and a failure to account for inflows of low-oxygen groundwater (Hall and Tank 2005). In addition, reach-scale methods incorporate both surface and hyporheic ER, but chambers isolate surface ER (Fellows et al. 2001). Previous estimates found that hyporheic ER can constitute 40-93% of measured reach-scale ER, depending on the amount of groundwater exchange (Fellows et al. 2001), and hyporheic exchange is typically highest in headwater streams (Anderson et al. 2005). Without knowing the hyporheic contribution to ER in streams at Coweeta, it is unknown whether our

estimates from chambers are comparable to the surface contribution of measured ER in previous whole-stream studies. Targeted experiments to understand the relative contribution of hyporheic and surface compartments to ER at Coweeta will improve our ability to link measurement scales and use chambers to understand whole-stream processes.

Utility of chamber-scale measurements in understanding stream function across a network

Chamber-based rates, and scaled rates based on chamber measurements, can be useful for quantifying ecosystem rates in sites where whole stream measurements are difficult. Streams within the Coweeta network have been noted as challenging for whole stream measurements due to high reaeration rates in shallow, high-gradient streams (e.g., Webster 2007, Benstead et al. 2009) and the potential for bias in metabolism estimates due to groundwater inflows (McCutchan et al. 2002, Hall and Tank 2005). If chambers are able to capture key drivers of rates that can be effectively scaled to the stream reach and network, chambers can be an effective tool in estimating surface metabolism and nutrient uptake, particularly in cold, high-gradient streams where direct whole-stream measurements are challenging.

Chambers can streamline ecosystem rate measurements and isolate drivers by reducing the effect of confounding factors at the reach scale, but understanding the limitations of their use is key. The challenges associated with using chambers to measure ecosystem processes are well-documented, and were succinctly summarized by Mulholland et al. (2001). Chief among these challenges are enclosure artifacts, difficulty including all representative substrates and ecosystem compartments in chambers, and accounting for spatial and temporal variability (Mulholland et al. 2001). In addition, studies have found that when chamber estimates are scaled-up to the stream reach based on reach-scale wetted area, chamber rates can both overestimate (Fellows et al.

2006) and underestimate (Grimm and Fisher 1984, McTammany et al. 2003, Fellows et al. 2006) whole-stream rates, and measurements of rates at both scales in single streams are few (but see Fellows et al. 2006). Here, we used reach temperature and light measurements to scale-up chamber rates. This approach also includes key assumptions, including that point measurements of stream light and temperature were representative of the reach as a whole. However, this scaling approach better accounts for differences in the magnitude and variability of temperature and light regime between the stream-side chamber and the whole reach than simply multiplying chamber rates up to a daily time step. Since chambers are typically run over short intervals during daytime hours, incorporating changes in light and temperature over longer time periods can substantially improve the relevance of chamber estimates to whole-stream rates.

Do different parts of the stream network contribute differently to ecosystem processes?

Stream size plays a role in the rate of biogeochemical processing in river networks. Our headwater network estimates suggest that for ER, GPP, and NH_4 uptake, 1st and 2nd order streams are hotspots of activity compared to larger 3rd and 4th order streams. Our estimates further emphasize the substantial role that the smallest streams play in nitrogen cycling, whether nitrogen uptake and retention is assessed through nitrogen tracer additions (Peterson et al. 2001, Mulholland et al. 2008), modeling (Seitzinger et al. 2002), or long-term datasets (Bernhardt et al. 2005). Across diverse watersheds, 1st and 2nd order streams are highly efficient at immobilizing nitrogen uptake due to high benthic surface area relative to water volume (Mulholland et al. 2008), and are estimated to retain ~20% of watershed nitrogen inputs (Seitzinger et al. 2002). Small, 1st and 2nd order streams are also important areas of organic matter storage and transformation, and their substantial contribution to total ER in headwater networks reflects their

short carbon turnover length (Webster 2007) and the importance of headwater streams in understanding global carbon emissions (Benstead and Leigh 2012, Hotchkiss et al. 2015). Because our scaled rates of ER and GPP were low compared to previously reported values from Coweeta, our estimates of the role of small streams in network carbon production and respiration are likely conservative. However, our estimates are also based on forested, retentive, and relatively unimpacted streams, and differences in land use among headwater streams across a heterogeneous landscape could contribute to variability in the relative contribution of 1st and 2nd order streams to rates of ER, GPP, and NH_4 uptake. Further improvements in mapping the extent of headwater streams (Benstead and Leigh 2012), tracking changes in land use and cover across the landscape, and reconciling chamber and reach-scale estimates will further refine our assessment of headwater contributions to network-scale carbon and nutrient cycling across heterogeneous landscapes.

Understanding how rates of metabolism and nutrient uptake change across relatively small gradients of stream size is important given the tenuous protection currently afforded small streams (Hawkins 2015). First and 2nd order streams are particularly vulnerable to degradation of habitat, water quality, and connectivity at present, given ongoing challenges to the Clean Water Rule at the state and federal level (Hawkins 2015). Compromised network connectivity through the loss of headwater streams or increased nutrient inputs in headwater catchments could alter biogeochemical processing at the network scale by shifting the balance of GPP, ER, and NH_4 uptake (Finlay 2011, Alexander 2015). Our work suggest that chambers can be useful to isolate drivers of ecosystem rates, and that consideration of seasonal and spatial variability is key to estimating of the role of different sized streams in headwater networks. Because all small

streams are not functionally equivalent, preserving the biogeochemical integrity of headwater networks will depend on protecting 1st and 2nd order streams.

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Supplementary Material

Appendix A. Chamber rates of ecosystem respiration (ER), gross primary production (GPP), and net ecosystem production (NEP) in April 2013 (E1 – E3) and September 2014 (all sites).

Table 2.1 Physical characteristics of study streams. Strahler stream order, elevation (Elev.), aspect, and upstream drainage area (Area) were quantified from a digital elevation model (DEM) with 60×60 m resolution, while distance downstream (Dist. down) was calculated from a raster file with 10 ×10 m resolution. Reach length indicates the length of stream used to quantify characteristics, and increased with stream size. Three streams were measured in April 2013, while 6 additional (9 total) streams were measured in September 2014. Photosynthetically active radiation (PAR) indicates daytime light availability, averaged (± 1 SE) between PAR meters at the top and bottom of each stream reach. Widths were measured at 11 transects along each stream reach; values represent mean ± 1 SE. Discharge was measured multiple times in each stream reach and values represent mean ± 1 SE. Sampled streams roughly correspond to the following numbered watersheds (WS) or weirs in the Coweeta Hydrologic Lab: E1 = WS 22, E2 = Weir 20, E3 = WS 9, S12 = WS 41, S16 = Weir 11, S17 = WS28, S19 = upper WS 28, S5 = WS8, and S9 = WS 7.

ID	Stream name	Order	Elev. (m)	Aspect	Area (km^2)	Dist. down. (km)	Reach length (m)	Month	PAR (µmol γ m^{-2} sec^{-1})	Width (m)	Discharge (L s^{-1})
E1	Lick Branch	2	886	NNE	0.32	0.80	50	April	68.96 ± 2.38	2.97 ± 0.11	27.11 ± 7.10
								Sept.	6.20 ± 0.19	2.03 ± 0.11	2.14 ± 0.16
E2	Middle Ball Creek	3	903	E	1.08	1.90	76	April	150.87 ± 4.47	4.01 ± 0.10	119.58 ± 20.94
								Sept.	24.67 ± 1.11	3.45 ± 0.15	17.55 ± 0.70
E3	Lower Ball Creek	4	727	NE	6.07	4.84	105	April	144.91 ± 4.96	5.47 ± 0.09	472.24 ± 8.80
								Sept.	40.31 ± 1.31	4.19 ± 0.10	89.88 ± 2.01
S5	Shope Fork	4	717	E	6.33	3.86	120	Sept.	27.59 ± 1.18	4.11 ± 0.16	102.60 ± 7.46
S9	Big Hurricane Branch	2	740	SSE	0.41	0.78	60	Sept.	5.98 ± 0.19	1.94 ± 0.11	5.61 ± 0.36
S12	Bates Branch	3	909	ESE	0.29	0.53	50	Sept.	9.69 ± 0.78	1.27 ± 0.08	3.35 ± 0.02
S16	Cunningham Creek	3	737	NE	0.28	2.67	70	Sept.	9.02 ± 0.31	3.03 ± 0.17	29.61 ± 0.87
S17	Henson Creek	3	994	E	1.43	2.10	70	Sept.	7.41 ± 0.22	2.26 ± 0.14	26.97 ± 1.66
S19	Unnamed Stream	1	1196	ENE	0.14	0.32	50	Sept.	11.69 ± 0.25	1.02 ± 0.14	1.87 ± 0.24

Table 2.2 Linear mixed effects models and parameter estimates for relationships between chamber rates (ecosystem respiration, gross primary production [mg O_2 m^{-2} min^{-1}]; ammonium uptake [µg N m^{-2} min^{-1}]) and hypothesized drivers (chamber water temperature [°C], coarse and fine benthic organic matter [CBOM, FBOM; g AFDM m^{-2}], photosynthetically active radiation [PAR; µmol photons m^{-2} s^{-1}], and chlorophyll-a [chl-a; mg m^{-2}]) from 9 streams (3: April 2013; 6 additional, 9 total: September 2014). Additional separate models for ammonium uptake were tested using ER, GPP, and water temperature as potential drivers. Model selection was based on AIC_C values. All models for ER and GPP included a nested random effect of stream within season to account for non-independence of replicate chamber runs (n = 5 – 10) within a stream and unquantified differences between seasons. Models of NH_4 uptake included a random effect of stream. Hypothesized drivers were uniquely quantified for each chamber run from a basket (CBOM, FBOM, chl-a), from a dissolved oxygen probe in the chamber during rate measurements (temperature), or from PAR recorded adjacent to each chamber during the net ecosystem production period of the chamber run. Hypothesized drivers and rates were natural log transformed to meet assumptions of normality. The number of parameters (K), AIC_C score, change in AIC_C from the top model (ΔAIC_C), weight and cumulative weight of support (W_i, $CumW_i$), log likelihood (LL), and conditional R^2 (R_c^2) are reported for models where $\Delta AIC_C \leq 2$ and are ranked by increasing AIC_C value, along with estimates of the intercept and slopes, standard errors (SE) and P-values. P-values in bold indicate statistical significance ($\alpha = 0.05$) while italicized P-values indicate marginal significance ($\alpha = 0.10$).

Model	**K**	**AIC_C**	**ΔAIC_C**	**W_i**	**$CumW_i$**	**LL**	**R_c^2**	**Estimate**	**SE**	**P**
Ecosystem respiration										
CBOM	4	167.28	0	0.29	0.29	-78.23	0.15			
Intercept								-1.97	0.24	**<0.001**
ln (CBOM)								0.22	0.06	**<0.001**
CBOM* × *Temperature	6	168.55	1.27	0.15	0.44	-76.49	0.19			
Intercept								-5.41	1.88	**0.005**
ln (CBOM)								1.28	0.59	**0.032**
ln (Temp.)								1.34	0.74	*0.073*
ln (CBOM) × ln (Temp.)								-0.40	0.22	*0.068*
FBOM* × *CBOM	6	168.92	1.64	0.13	0.57	-76.67	0.19			
Intercept								-2.94	0.60	**<0.001**
ln (FBOM)								0.31	0.17	*0.079*
ln (CBOM)								0.48	0.20	**0.018**
ln (FBOM) × ln (CBOM)								-0.08	0.05	0.105

CBOM + FBOM	5	169.16	1.88	0.11	0.68	-77.99	0.16			
Intercept								-2.05	0.27	**<0.001**
ln (CBOM)								0.18	0.08	**0.024**
ln (FBOM)								0.05	0.08	0.495
Gross primary production										
PAR	5	13.16	0	0.67	0.67	-2.67	0.35			
Intercept								-0.003	0.12	0.985
ln (PAR)								0.10	0.03	**0.008**
Ammonium uptake										
FBOM + CBOM + Chl-a + PAR	7	524.51	0	0.99	0.99	-253.74	0.12			
Intercept								-101.78	102.74	0.327
ln (FBOM)								24.35	18.33	0.191
ln (CBOM)								18.93	12.22	0.128
ln (Chl-a)								-0.46	10.54	0.966
ln (PAR)								-14.13	13.94	0.316
ER + GPP	5	602.51	0	0.56	0.56	-295.59	0.06			
Intercept								125.07	41.13	**0.004**
ln (ER)								47.52	31.71	0.140
ln (GPP + 1)								-59.79	82.72	0.473
ER + GPP + Temperature	6	603.04	0.53	0.43	0.99	-294.57	0.10			
Intercept								872.26	518.63	*0.099*
ln (ER)								60.83	32.42	*0.066*
ln (GPP + 1)								-125.29	92.89	0.183
ln (Temp.)								-252.62	174.81	0.155

Table 2.3 Linear mixed effects models and parameter estimates for relationships between chamber rates (ecosystem respiration, gross primary production [mg O_2 m^{-2} min^{-1}]; NH_4 uptake [µg N m^{-2} min^{-1}]) scaled to whole stream temperature and light and distance downstream (km), aspect, and season (spring/fall) from 3 (April 2013) or 9 (September 2014) streams. Model selection was based on AIC_C values. All models included a random effect of stream to account for non-independence of replicate chamber runs (n = 5 – 10) within a stream each season. Distance downstream was calculated as distance from the headwaters using a stream network raster file with 10 ×10 m resolution. Scaled rates were natural log transformed to meet assumptions of normality. Parameter definitions are as listed in Table 2.2.

Model	K	AIC_C	ΔAIC_C	W_i	$CumW_i$	LL	R_c^2	Estimate	SE	P
Ecosystem respiration										
Season	4	169.54	0	0.65	0.65	-80.50	0.05			
Intercept								-1.05	0.15	**<0.001**
Season								0.36	0.18	**0.047**
Distance downstream + season	5	171.44	1.90	0.25	0.90	-80.31	0.05			
Intercept								-1.14	0.21	**<0.001**
Distance downstream								0.03	0.05	0.539
Season								0.38	0.18	**0.039**
Gross primary production										
Distance downstream × season	6	-87.36	0	0.87	0.87	50.24	0.99			
Intercept								-2.87	0.30	**<0.001**
Distance downstream								-0.05	0.12	0.694
Season								-2.60	0.06	**<0.001**
Distance downstream × season								0.46	0.02	**<0.001**
Ammonium uptake										
Distance downstream	4	617.50	0	0.94	0.94	-304.32	0.02			
Intercept								85.79	20.74	**<0.001**
Distance downstream								-8.29	7.91	0.300

Table 2.4 Estimates of the contribution of 1st – 4th order streams to network-scale rates of GPP, ER, and NH_4 uptake. Total stream length and streambed area per order are from Rosemond et al. (2015). Network rates of ER and GPP (kg C d^{-1}) were calculated using the mean of scaled rate estimates and streambed area; network NH_4 was from mean chamber uptake rates and streambed area. Negative values of NH_4 uptake indicate net mineralization; positive values indicate net uptake. Values of length and streambed area in parentheses indicate cumulative proportions. Values of network rates in parentheses indicate minimum and maximum daily estimates based on the 95% confidence interval of mean daily rates. % ER, % GPP, and % NH_4 are the estimated proportion of the total mean daily rate among 1st – 4th order streams provisioned by each stream order.

Order	Length (km)	Streambed area (km^2)	Network ER (kg C d^{-1})	Network GPP (kg C d^{-1})	Network uptake (kg N d^{-1})	% ER	% GPP	% NH_4
1	2001.0 (56.2%)	2.42 (42.5%)	794 (0[a], 1932)	9 (NA[b])	335 (47, 622)	57.5	23.6	53.2
2	886.6 (81.1%)	1.35 (66.3%)	255 (<1, 357)	10 (5, 16)	101 (22, 180)	18.5	26.6	16.1
3	452.7 (93.9%)	1.08 (85.2%)	180 (<1, 248)	8 (4, 11)	138 (68, 208)	13.0	19.9	22.0
4	218.3 (100.0%)	0.84 (100.0%)	152 (<1, 184)	12 (9, 14)	55 (-11[c], 120)	11.0	29.9	8.7

a. Lower CI estimate for ER was <0, which is not biologically possible.
b. Chambers only run in one 1st order stream, thus there was only one estimate of daily GPP and no min. or max. values are presented.
c. Negative NH_4 uptake represents net mineralization.

Figure Legends

Fig. 2.1. Simplified diagram of streams in the Coweeta Creek network within the US Forest Service Coweeta Hydrologic Laboratory. Filled circles (n = 3) indicate sites where ecosystem rates were measured in both April 2013 and September 2014. Open circles (n = 6) indicate sites measured in September 2014 only. Inset shows the southeastern USA; the black circle on the inset indicates the stream network location.

Fig. 2.2. Changes in (A) water temperature, (B) light availability (PAR) at the stream surface, and (C) coarse and (D) fine benthic organic matter across the stream network and between seasons (April 2013: filled circles; September 2014: open circles). Temperature and PAR were logged every 10 minutes at the most downstream sampling point of each stream. Benthic organic matter was quantified from baskets incubated in the stream for ~30 days. Points represent mean values for each sampled stream ± 1 SE. Solid lines indicate significant ($\alpha = 0.05$) changes across the network; dashed lines indicate marginal ($\alpha = 0.10$) changes.

Fig. 2.3. Predicted drivers of ecosystem respiration (ER), gross primary production (GPP), and ammonium (NH_4) uptake rates. Predicted drivers for ER were (A) coarse (CBOM) and (B) fine (FBOM) benthic organic matter; for GPP, (C) photosynthetically active radiation (PAR) and (D) chlorophyll *a*; and for NH_4 uptake, (E) chamber ER and (F) chamber GPP. All rates were measured using recirculating chambers run in April 2013 (filled circles) and September 2014 (open circles). Points represent mean values for each sampled stream ± 1 SE. Solid lines indicate significant ($\alpha = 0.05$) fits from one or more top ($\Delta AIC_C \leq 2$) linear mixed effects models (Table 2.3); dashed lines indicate marginal ($\alpha = 0.10$) fits. In (E) and (F), dotted lines indicate zero net

uptake; negative values indicate net NH_4 mineralization and positive values indicate net NH_4 uptake. Note that axes (except y-axis in E, F) are natural log transformed. Ranges of site means of back transformed logged values are: ER (0.19 – 1.17 mg O_2 m^{-2} min^{-1}), GPP (0.03 – 0.95 mg O_2 m^{-2} min^{-1}), CBOM (13.45 – 283 g AFDM m^{-2}), FBOM (8.68 – 284 g AFDM m^{-2}), PAR (2.43 – 213 µmol photons m^{-2} sec^{-1}), chl-a (0.16 – 4.00 mg m^{-2}).

Fig. 2.4. Changes in estimated whole-stream daily rates of (A) ER, (B) GPP, and (C) NH_4 uptake as a function of distance downstream based on scaling chamber rates to reach-scale temperature and light regime. Points indicate mean (± 1 SE) rates per stream in April 2013 (filled circles) and September 2014 (open circles). There was a significant ($\alpha = 0.05$) increase in GPP with distance downstream in September ($\ln(y) = 0.42x - 5.47$). Note that y-axes in (A) and (B) are natural log transformed, but axis labels indicate non-transformed values for ease of interpretation.

Fig. 2.5. Estimated rates of reach-scale ecosystem respiration (ER) and gross primary production (GPP) based on measurements in recirculating chambers in April 2013 (filled black circles) and September 2014 (open circles). Scaled rates are based on reach-scale temperature and light regime. Filled gray circles are previously published values of ER and GPP measured at the reach scale in Coweeta streams (Mulholland et al. 1999, Bernot et al. 2010, Hagen et al. 2010, Northington et al. 2013). Rates were significantly correlated across all data; Pearson correlation $r(104) = 0.59$, $p < 0.001$. Dashed lines indicates P:R 1:1 line with net heterotrophy (P:R < 1) above the line and net autotrophy (P:R > 1) below.

Fig. 2.1

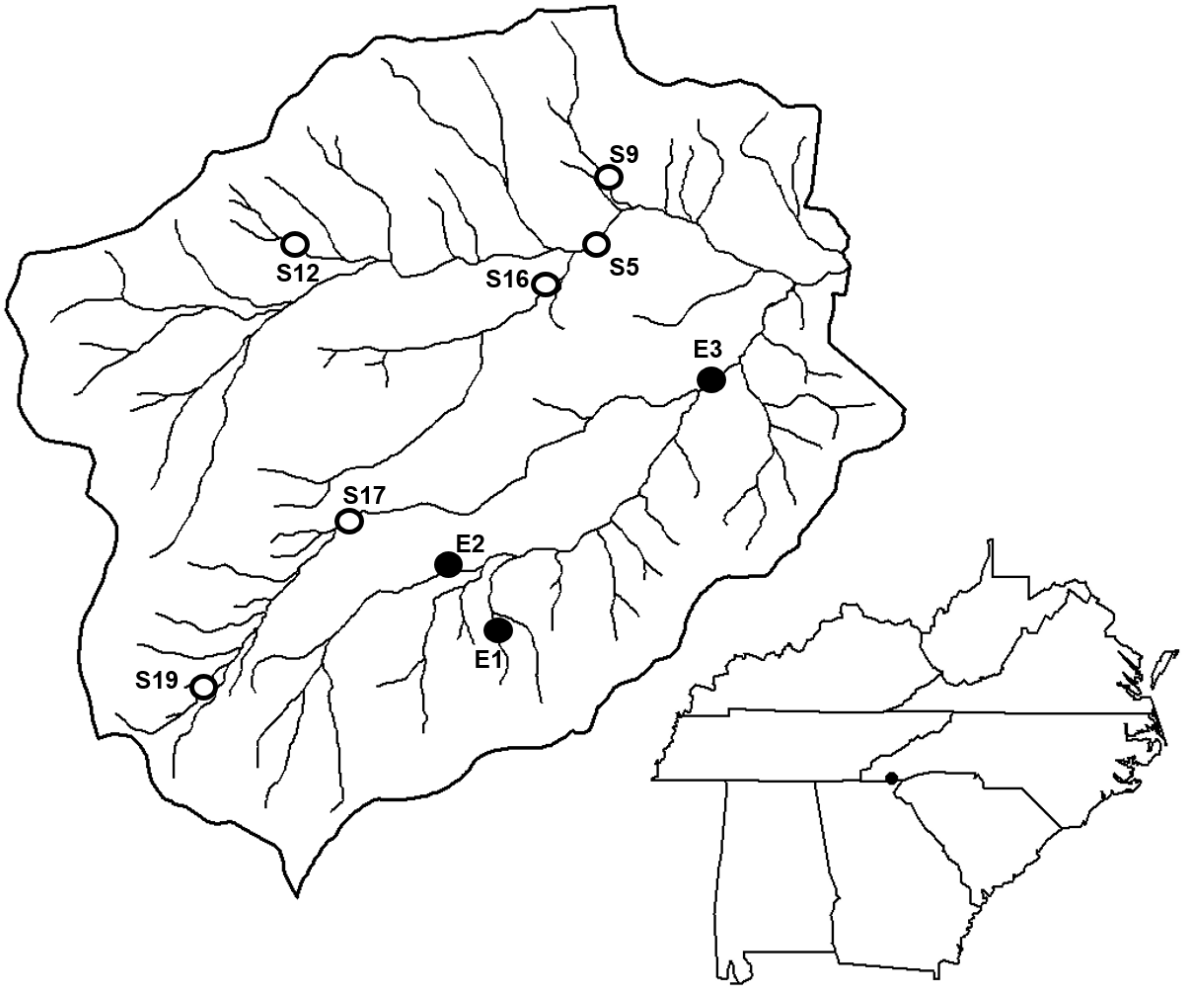


Fig. 2.2

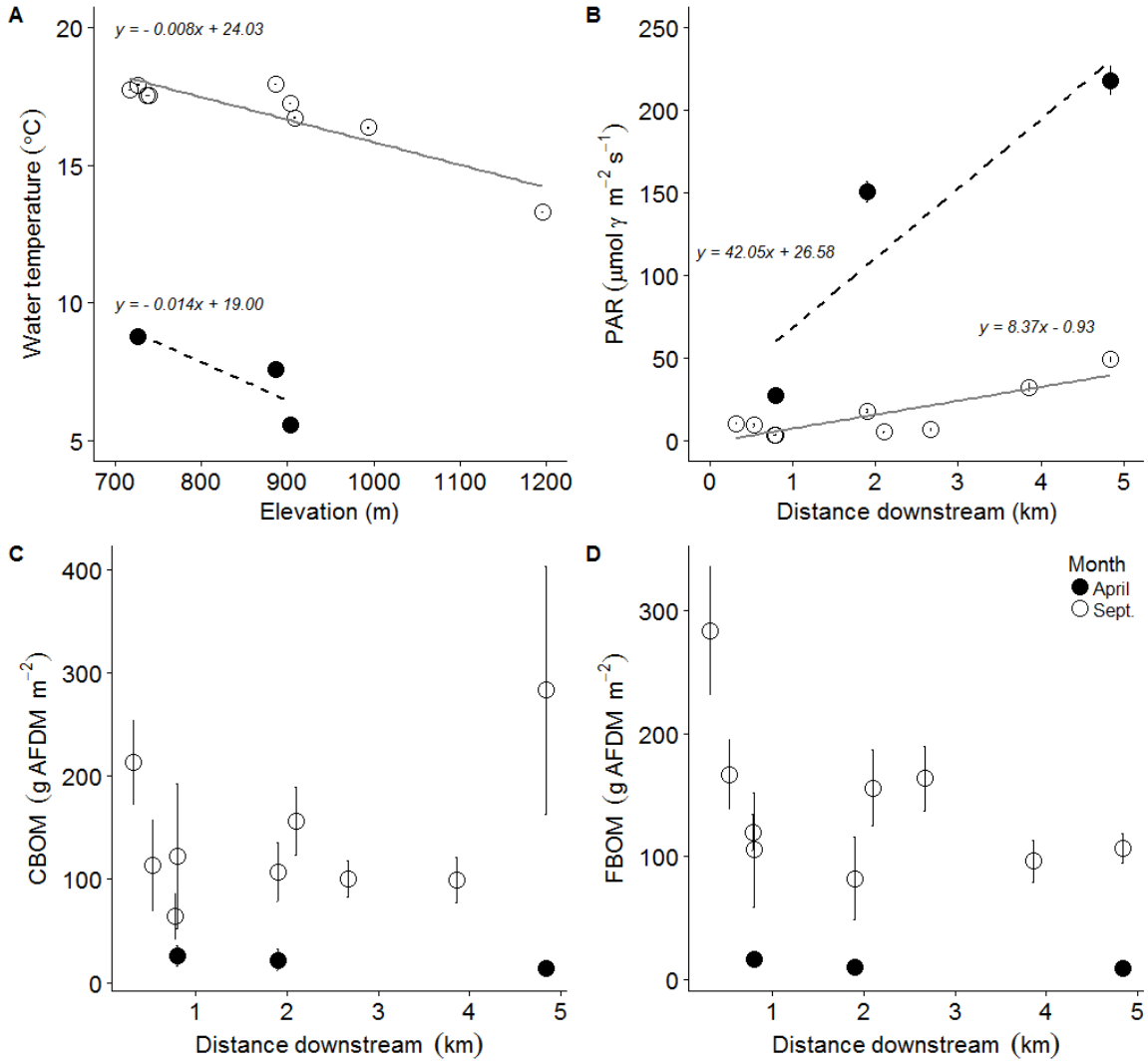

A
y = - 0.008x + 24.03
y = - 0.014x + 19.00
Water temperature (°C)
Elevation (m)
B
y = 42.05x + 26.58
y = 8.37x - 0.93
PAR (µmol γ m-2 s-1)
Distance downstream (km)
C
CBOM (g AFDM m-2)
Distance downstream (km)
D
FBOM (g AFDM m-2)
Distance downstream (km)
Month
April
Sept.

Fig. 2.3

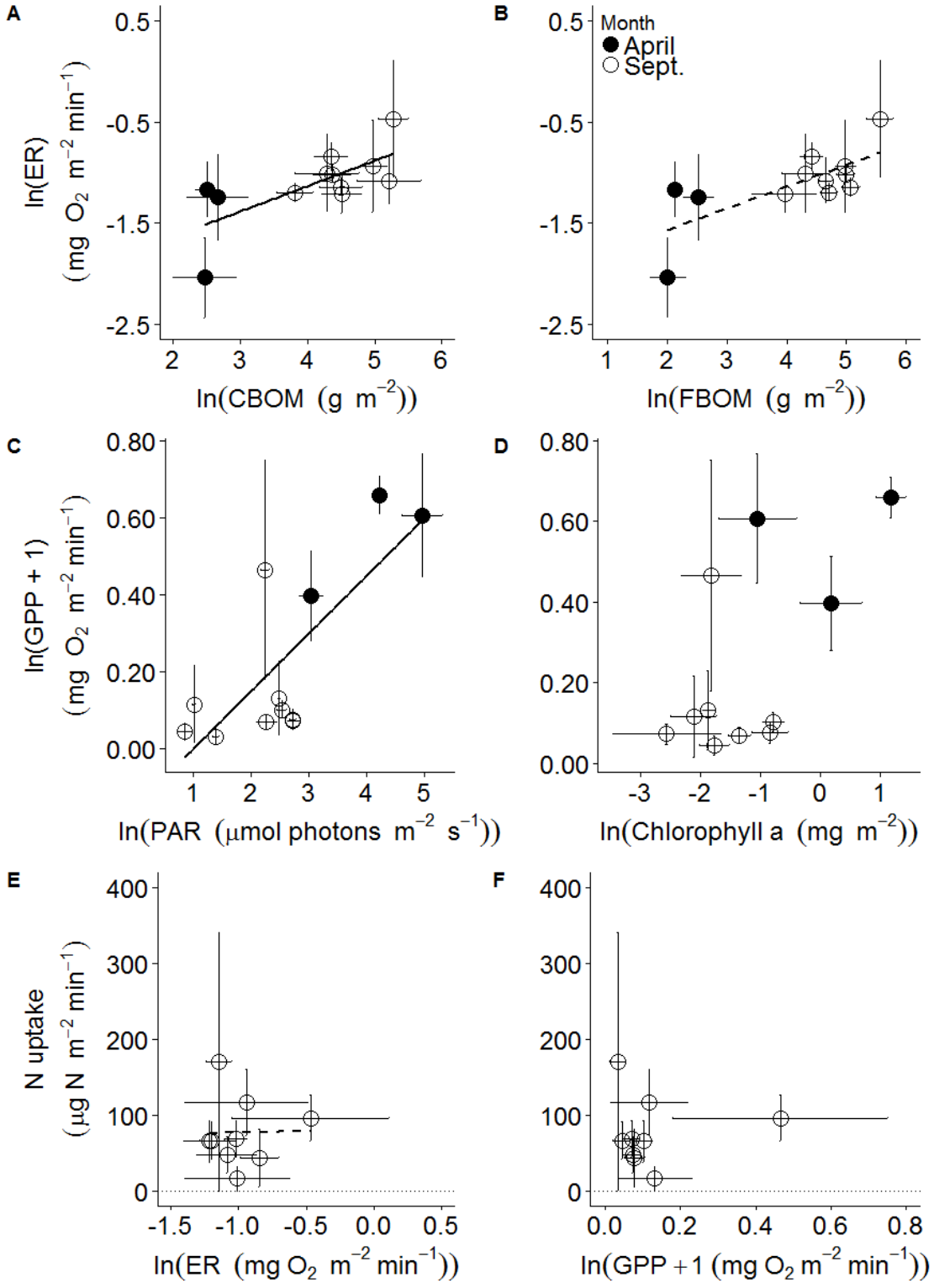

A
B
C
D
E
F
Month
April
Sept.
ln(ER) (mg O2 m−2 min−1)
ln(CBOM (g m−2))
ln(FBOM (g m−2))
ln(GPP + 1) (mg O2 m−2 min−1)
ln(PAR (μmol photons m−2 s−1))
ln(Chlorophyll a (mg m−2))
N uptake (μg N m−2 min−1)
ln(ER (mg O2 m−2 min−1))
ln(GPP + 1 (mg O2 m−2 min−1))

Fig. 2.4

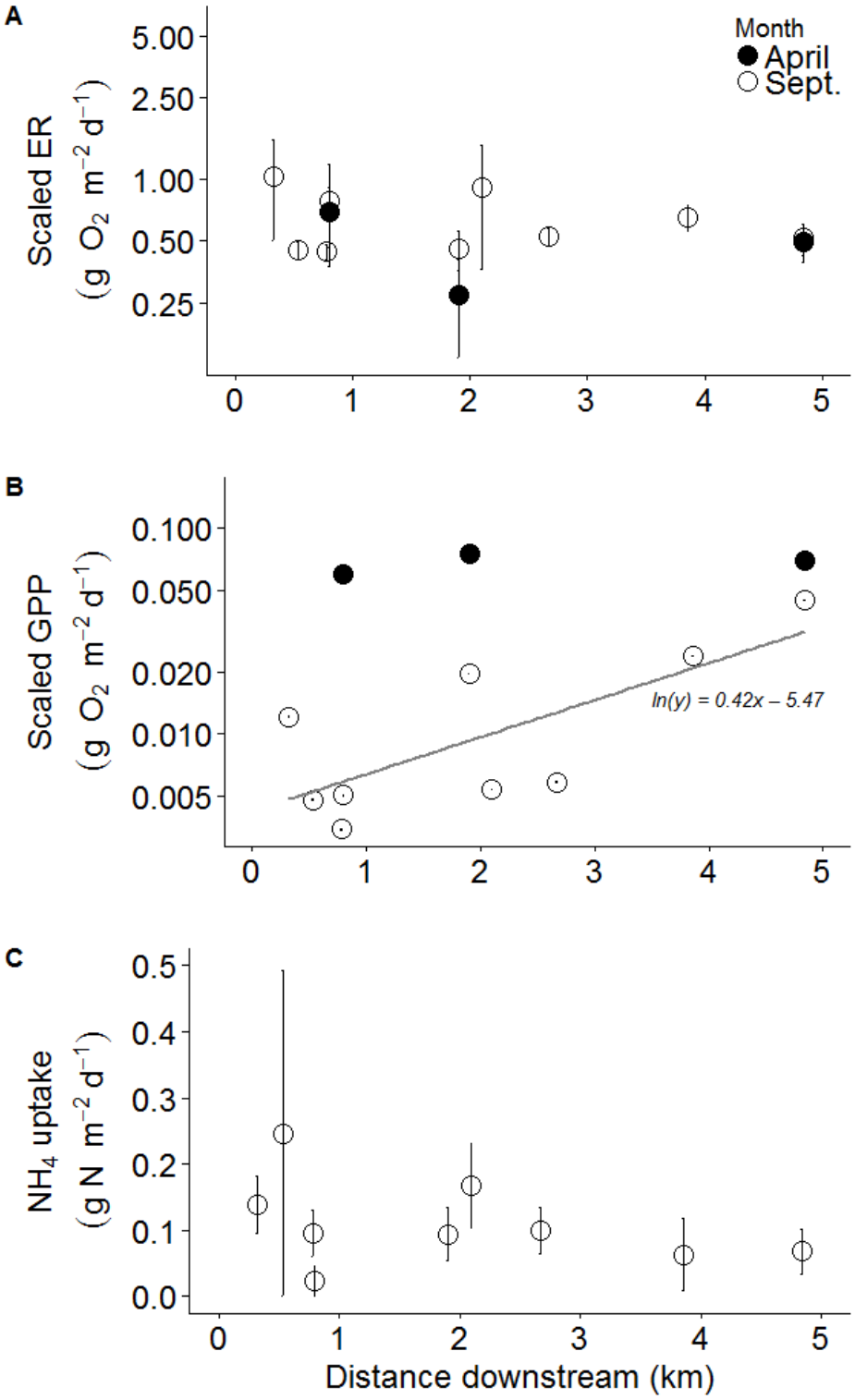
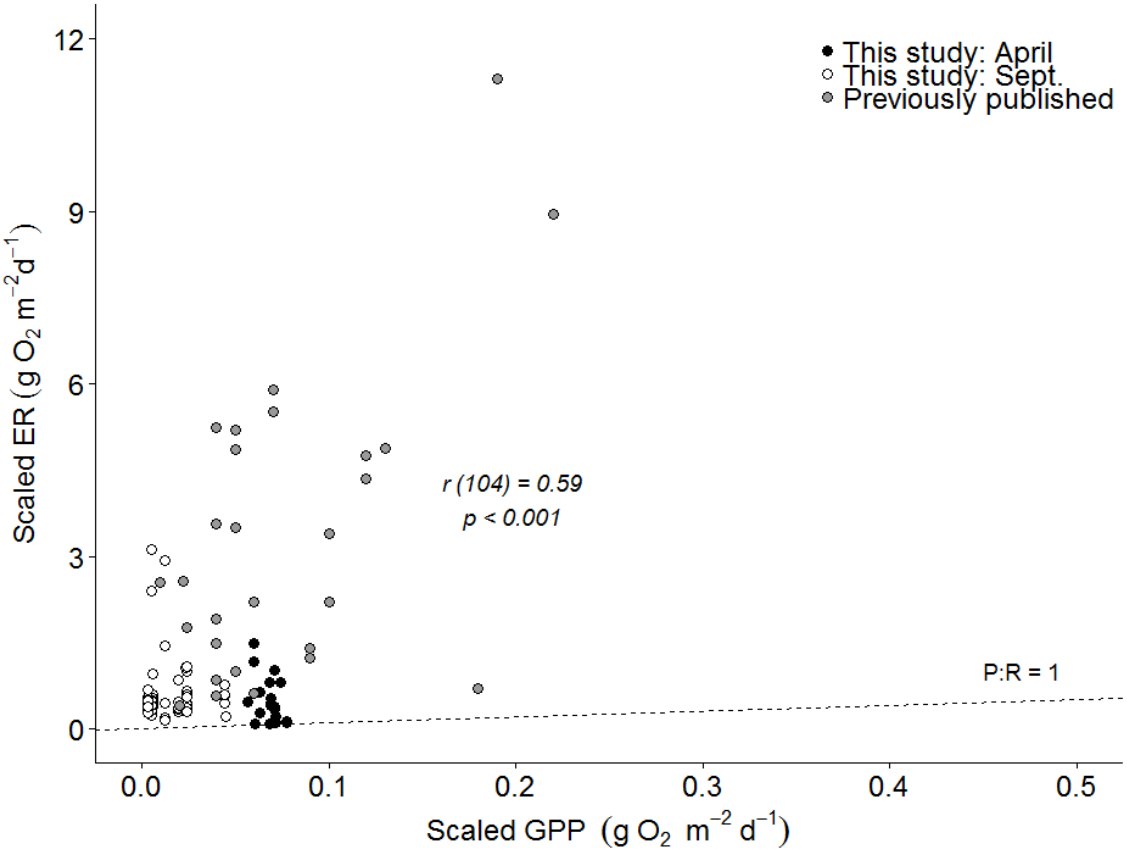

A
Month
April
Sept.
Scaled ER
(g O2 m-2 d-1)
5.00
2.50
1.00
0.50
0.25
0
1
2
3
4
5
B
Scaled GPP
(g O2 m-2 d-1)
0.100
0.050
0.020
0.010
0.005
ln(y) = 0.42x – 5.47
C
NH4 uptake
(g N m-2 d-1)
0.5
0.4
0.3
0.2
0.1
0.0
Distance downstream (km)

Fig. 2.5


This study: April
This study: Sept.
Previously published
r (104) = 0.59
p < 0.001
P:R = 1
Scaled ER (g O2 m-2d-1)
Scaled GPP (g O2 m-2 d-1)
0
3
6
9
12
0.0
0.1
0.2
0.3
0.4
0.5

CHAPTER 3

DETRITAL RESOURCE STOICHIOMETRIC C:N:P BASELINES IN STREAM ECOSYSTEMS[2]

[2] Farrell, K.J., A.D. Rosemond, F. Ballantyne IV, J.S. Kominoski, S.M. Bonjour, J. Rüegg, L.E. Koenig, C.L. Baker, M.T. Trentman, K.R. Sheehan, and T.K. Harms. To be submitted to *Ecosystems*.

Abstract

Stoichiometric ratios have been used to predict nutrient limitation in diverse ecosystems. In streams, coarse and fine benthic organic matter (CBOM, FBOM) fuel food webs, but little is known about how resource quality of these two pools differs within stream networks and across stream networks from different biomes. We measured carbon (C), nitrogen (N), and phosphorus (P) content of CBOM and FBOM from multiple locations in stream networks in tropical forest, temperate deciduous forest, tallgrass prairie, and boreal forest biomes, and compared differences in the magnitude and variability of elemental stoichiometric ratios (C:N, C:P, N:P) within and across those stream networks. Across biomes, CBOM had consistently higher C:N and C:P, and was more variable than FBOM. The lower and less variable C:N and C:P ratios of FBOM suggest that microbial processing of CBOM results in tightly constrained resources despite large initial differences in CBOM stoichiometry. In contrast, % P and N:P exhibited biome-specific differences between the two resource pools, with higher % P in FBOM in tropical and temperate deciduous biomes, and no significant differences in N:P in the grassland or boreal forest biomes. When compared to potential terrestrial source materials, CBOM C:N and C:P ratios were similar to terrestrial foliage, which indicates that in-stream microbial conditioning of CBOM may compensate for nutrient resorption from foliage prior to leaf-fall. Based on observed stoichiometric ratios, aquatic detritivores that feed on CBOM (shredders) may be more likely to experience nutrient (N and/or P) limitation across sampled stream networks, whereas consumers of FBOM (collectors) may be more vulnerable to C limitation. Considering the implications of consumer-resource stoichiometric imbalances on carbon and nutrient cycling is an important next step in understanding the biogeochemical implications of stoichiometric differences of basal resources in streams.

Introduction

Ecological stoichiometry uses the balance of carbon (C) and other macroelements (here nitrogen [N] and phosphorus [P]) to understand ecosystem processes (Sterner and Elser 2002), and has been used as a mechanistic framework to assess nutrient dynamics in multiple ecosystems (Elser et al. 2007, Hessen et al. 2013). A stoichiometric approach has been particularly useful in evaluating relationships between resources and consumers, as interactions between organisms and their environment are influenced by their elemental demands relative to elemental supplies (Elser and Urabe 1999). Indeed, elemental mass and stoichiometric imbalances between consumers and their resources can constrain consumer growth and in turn affect resource processing (Dodds et al. 2004, Frost et al. 2005, Cebrian et al. 2009). For example, increased detrital carbon:nitrogen (C:N) ratios have been linked to reduced litter breakdown rates due to nutritional imbalances for stream detritivores (Hladyz et al. 2009), and can reduce N uptake rates in streams through decreased N mineralization (Dodds et al. 2004). To predict effects of stoichiometric imbalances between consumers and their resources on ecosystem processes, threshold elemental ratios (TERs; Sterner and Elser 2002) have been quantified, which describe the resource ratio below which a consumer will be carbon limited and thus excreting macroelements and above which it will be nutrient-limited (Frost et al. 2006). Combining basal resource stoichiometry with consumer TERs thus provides a platform from which to predict rates of nutrient cycling and constraints on organismal growth.

While the stoichiometry of consumers and thus their TERs are fairly constrained (but see Halvorson and others 2015), basal resources can be highly variable within and across ecosystems, and their elemental ratios are not yet fully understood in any system, including detritus-based streams. Data on basal resource nutrient content and calculated stoichiometric

ratios (e.g., carbon:nitrogen [C:N], carbon:phosphorus [C:P], nitrogen:phosphorus [N:P]) have been collected from terrestrial leaves and litters (Cebrian et al. 1998, 2009, McGroddy et al. 2004), soils (Cleveland and Liptzin 2007), and marine and freshwater primary producers and detritus (Cebrian et al. 1998, 2009, Elser et al. 2000, Dodds et al. 2004). Synthesis efforts using these data have assessed broad-scale differences between basal resources in terrestrial, marine, and lacustrine environments, and found both similarities and differences in the stoichiometric ratios of basal resources within and across ecosystems. For example, a global synthesis of terrestrial green foliage and litters found that while the variability of C:N:P was tightly constrained within biomes, there were significant biome-level differences in resource stoichiometry; C:P and N:P were significantly higher in tropical than temperate forests, though C:N did not differ between the two biomes (McGroddy et al. 2004). Also, in a cross-system comparison, Elser and others (2000) found that despite terrestrial foliage having much higher C:N and C:P ratios than lake seston, the N:P ratios of terrestrial and lacustrine autotrophs were nearly identical. Largely missing from these syntheses is an assessment of basal resource stoichiometry within and across detritus-based lotic ecosystems (streams, rivers), though stoichiometric ratios have been previously reported in site-specific stream studies (e.g., Cross and others 2003; Dodds and others 2004; Evans-White and others 2009; Cheever and others 2013).

Streams are often net heterotrophic (Mulholland et al. 2001) and to better understand carbon and nutrient dynamics in these detritus-based ecosystems, knowledge of the stoichiometry of benthic organic matter, a main resource for stream food webs, is needed. Quantifying differences in the carbon:nutrient ratios of coarse and fine benthic organic matter can provide insights into potential nutrient availability in these systems, and cross-system

comparisons can help determine the generality of stoichiometric changes between these detrital pools. Changes in the carbon to nutrient ratios of leaf detritus in streams due to microbial conditioning and breakdown are well documented, with both C:N and C:P decreasing with time in the stream as microorganisms colonize and process leaf material (e.g., Manning et al. 2016). However, less is known about how the stoichiometry of detritus differs between carbon pools (coarse [leaf] and fine benthic carbon) within and between stream networks (but see Cross and others 2005). Only if we can understand the natural variation in the elemental concentration and ratios of organic matter can we then make specific predictions about how basal resource nutrient content and stoichiometry may change in future climate and land use scenarios (Kominoski and Rosemond 2012). In lotic environments, understanding the foundational patterns of how resource nutrient content and stoichiometry vary among mostly undisturbed stream networks can provide insights into how these systems could be altered by ongoing changes in climate and land use or with increased nutrient pollution.

Here, we present data on the stoichiometry of two compartments of basal carbon in streams: coarse and fine benthic organic matter (hereafter CBOM and FBOM), collected throughout four river networks in distinct biomes. To assess how the spatial extent of sampling may affect our understanding of the stoichiometry of each detrital pool, we tested whether variability in resource nutrient content and stoichiometry increased as the spatial scale of sampling increased (e.g., from a single stream to a network to different biomes). We predicted that the variability of resource nutrient content and stoichiometry would be significantly greater across biomes than within individual streams or networks. We tested for differences in nutrient content and stoichiometry of CBOM and FBOM, to determine whether in-stream processing leads to a convergence in basal resource quality and to compare potential nutrient constraints for

different macroinvertebrate consumers. We hypothesized there would be biome-level differences in CBOM stoichiometry due to differences in riparian vegetation and corresponding litter nutrient chemistry, but expected FBOM would be more consistent across biomes, as microbial colonization and processing would reduce differences across biomes (Findlay et al. 2002), resulting in overall higher and less variable quality (lower and less variable C:N and C:P) in FBOM than CBOM. To determine whether shredders feeding on CBOM were likely more N and/or P limited than collectors feeding on FBOM, we compared our resource nutrient content and stoichiometry to previously published TERs of stream detritivores. Finally, we compared our stoichiometry data to terrestrial (foliage, soil) and lacustrine (seston) ecosystems (Elser et al. 2000, McGroddy et al. 2004, Cleveland and Liptzin 2007), to assess whether the stoichiometric ratios of stream basal resources are similar to resources in forests and lakes, which would indicate a generality of resource content available to primary consumers in aquatic and terrestrial ecosystems.

Methods

Study sites

We sampled basal resources from streams in four relatively undisturbed networks that represented distinct biomes: tropical forest, temperate deciduous forest, tallgrass prairie, and boreal forest (Table 3.1, Fig. 3.1). The four stream networks were affiliated with the Luquillo (LUQ), Coweeta (CWT), Konza Prairie (KNZ), and Bonanza Creek (CPC) Long Term Ecological Research (LTER) sites, respectively. The Río Mameyes (LUQ) is a fifth-order network in northeastern Puerto Rico that drains 17.8 km^2 of tropical forest and is within the study area of the Luquillo LTER. Streams are heavily shaded by a canopy dominated by tabonuco

(*Dacryodes excelsa*) and sierra palm (*Prestoea montana*; Benstead and others 2010). Coweeta Creek (CWT) is a fifth-order network draining 14.4 km^2 in the Southern Appalachian Mountains in North Carolina within the Coweeta LTER site. Streams are heavily shaded by an overstory of temperate mixed hardwood species (primarily oak [*Quercus* spp.], tulip poplar [*Liriodendron tulipifera*], and red maple [*Acer rubrum*]) and a dense understory of *Rhododendron maximum* (Swank and Crossley 1988). Kings Creek (KNZ) is a fourth-order network draining 13.1 km^2 of tallgrass prairie within the Konza Prairie LTER in the Flint Hills of Kansas. Streams are intermittent and flood seasonally. Riparian vegetation throughout the network consists of gallery forests of oak (*Quercus macrocarpa, Q. muehlenbergii*), hackberry (*Celtis occidentalis*), and elm (*Ulmus americana*), and C_4 prairie grasses (e.g., *Andropogon gerardii, A. scoparius, Sorghastrum nutans;* Gray 1997; Veach and others 2014). Caribou Creek (CPC) is a third-order network draining 60.8 km^2 of boreal forest in interior Alaska, within the study area of the Bonanza Creek LTER. Streams are underlain by discontinuous permafrost, and riparian vegetation is dominated by dwarf shrubs (birch [*Betula glandulosa*], cranberry [*Vaccinium vitis-idaea* and *V. oxycoccus*], and blueberry [*Vaccinium uliginosum*]) and tussock-forming grasses and sedges (*Eriophorum* spp. and *Calamagrostis canadensis*; Haugen et al. 1982).

Sampling occurred between February 2013 and March 2014 as part of the Scale, Consumers, and Lotic Ecosystem Rates (SCALER) project (Table 3.1). Sampled streams within each network represented a range of stream sizes to capture gradients in discharge, elevation, and upstream drainage area (Table 3.1). Discharge was measured at each stream by releasing a slug of conservative tracer (sodium chloride [NaCl] or sodium bromide [NaBr]) and monitoring specific conductance (for NaCl) or bromide concentration with an ion specific probe (for Br^-) at the downstream station of each delineated stream reach (Kilpatrick and Cobb 1985). Dissolved

inorganic nitrogen (DIN: nitrate [NO_3] and ammonium [NH_4]) and soluble reactive phosphorus (SRP) were analyzed using standard methods (APHA 2005) on water samples that were filtered (0.7 µm glass fiber) and frozen until analysis. Upstream drainage area, streambed slope, and elevation of study streams were calculated from a high-resolution digital elevation model of each drainage basin. Coordinates of the most downstream point of each study reach were combined with a flow accumulation grid to calculate upstream drainage area (K. Sheehan, unpublished data).

Organic matter elemental mass, stoichiometry, and isotopes

Samples of coarse (>1 mm, CBOM) and fine (<1 mm, FBOM) benthic organic matter were collected randomly from up to four blocks distributed within each sampled stream reach. CBOM, which was a composite sample of terrestrially-derived material (e.g., conditioned leaves, twigs, and small wood), was collected from the benthic surface by hand and stored in a polyethylene bag. FBOM was collected from the benthic surface using a 50-mL bulb syringe, or was subsampled after agitating surface sediments enclosed in an open-bottom bucket and stored in a 250-mL polyethylene bottle. All samples were stored on ice after collection and frozen (-20°C) upon return to the laboratory.

All samples were processed at the University of Georgia Analytical Chemistry Lab (Athens, GA). Samples of both CBOM and FBOM were freeze-dried, then ground to a fine powder using a ball mill (SPEX SamplePrep, Metuchen, New Jersey, USA). Subsamples of each resource type (CBOM, FBOM) were analyzed for total C and N, as well as isotopic ratios ($\delta^{13}C$, $\delta^{15}N$), with a CHN elemental analyzer (Carlo Erba NA-1500, Milan, Italy) coupled to a Thermo Delta V isotope ratio mass spectrometer via a Thermo Conflo III interface (Thermo Fisher

Scientific, Bremen, Germany). Phosphorus content of subsamples was analyzed colorimetrically following acid digestion (APHA 2005) using a spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) at 640 μm wavelength. Percent elemental content (% C, %N, % P) was calculated based on sample dry mass (CBOM: ~100 mg, FBOM: ~25 mg). All elemental ratios are presented in molar units. Stable isotope signatures of CBOM and FBOM were calculated based on deviation from a standard (δ^{13}C: Pee Dee Belemnite; δ^{15}N: atmospheric N_2) using the following equation: $\delta X(‰) = \left(\frac{\delta X_{sample} - \delta X_{standard}}{\delta X_{standard}}\right) \times 1000$, where X is ^{13}C or ^{15}N (Peterson 1999). Repeatability was ± 0.08 ‰ for δ^{13}C and ± 0.10 ‰ for δ^{15}N.

Consumer threshold elemental ratios

We compared resource nutrient content to the nutritional requirements of macroinvertebrate shredders, which consume CBOM, and collectors, which consume FBOM, using detritivore C:N and C:P TERs estimated by Tant and others (2013). A range for each TER was calculated based the standard error of consumer body stoichiometry reported by Cross and others (2003). While this approach is limited in that TERs were estimated based on macroinvertebrates from a single location (CWT, Tant and others 2013), previous work assessing changes in macroinvertebrate body stoichiometry suggest that there is little regional variation within taxonomic groups (Evans-White et al. 2005). We compared C:N and C:P TERs to the stoichiometric ratios of CBOM and FBOM samples to estimate whether stream detritivores in each biome were likely C or nutrient (N and/or P) limited (e.g., CBOM ratio $_{[C:N \text{ or } C:P]} > TER_{shredder}$ = N or P limitation; FBOM ratio $_{[C:N \text{ or } C:P]} < TER_{collector}$ = C limitation). Despite some limitations, TER estimates provide an approximation of potential nutritional limitations for stream macroinvertebrates based on resource nutrient content.

Statistical analyses

We used linear mixed effects models ('nlme' package in R; Pinheiro and others 2016) to test whether there were consistent differences in nutrient content (% C, N, P), stoichiometry (C:N, C:P, N:P), and isotope values between CBOM and FBOM within and among biomes. Models included fixed effects of biome, sample type (CBOM, FBOM), and their interaction, and stream within a biome as a random effect to account for multiple samples from each stream. Post hoc comparisons between biomes, and between resource types within a biome, were conducted based on least-squares means using the 'lsmeans' package (Lenth 2016).

To assess whether variability in nutrient content and stoichiometry of each resource pool increased with the spatial extent of sampling (stream, network, cross-biome), we calculated a coefficient of variation (CV) for each resource pool for each nutrient (% C, N, P) and ratio (C:N, C:P, N:P). Within-stream CVs were calculated separately for CBOM and FBOM (n = 3 – 4 replicates each) within each sampled reach. Within biome (network) CVs were calculated using the reach-scale mean of nutrient content or ratio for each resource pool (CBOM, FBOM). Across-biome CVs were calculated based on the mean nutrient content or ratio for CBOM and FBOM of each biome. We assessed whether variability increased across spatial scales (reach to network, network to cross-biome) for each nutrient or ratio using ANOVA with an interaction between basal resource (CBOM or FBOM) and spatial extent (within stream, within network, cross-biome). Post-hoc tests of significant effects were conducted using 'lsmeans'. All analyses were conducted using R 3.3.3 (R Core Team 2017).

Results

Organic matter elemental mass, stoichiometry, and isotopes

We found that CBOM had consistently higher C:nutrient stoichiometry than FBOM, though there were biome-specific differences in the degree of difference between CBOM and FBOM nutrient content and stoichiometry (Table 3.2, Appendix B). Across all biomes, CBOM had significantly higher % C than FBOM (Table 3.2; Fig. 3.2A). In all four biomes, CBOM had 5-9× higher % C than FBOM, with larger differences between CBOM and FBOM at LUQ than in other biomes (Fig. 3.3A). Percent N was also consistently higher in CBOM than FBOM (Table 3.2, Fig. 3.2B), with 1.5-2.5× higher % N in CBOM than FBOM (Fig 3.3B, Appendix B). Globally (i.e., across all samples), the two resource types did not differ in % P (Table 3.2; Fig. 3.2C), though there were biome-specific patterns in % P differences between CBOM and FBOM (Fig. 3.3C). In KNZ and CPC, % P was 2-3× higher in CBOM than FBOM, while in LUQ and CWT, % P was higher in FBOM than CBOM (Fig. 3.3C).

Differences in % C between CBOM and FBOM resulted in significantly higher C:N and C:P ratios in CBOM than FBOM (Table 3.2; Fig. 3.2D-E). Across all biomes, CBOM C:N was 2.5-4× greater than FBOM C:N (Fig. 3.3D), and CBOM C:P was 4-13× greater than FBOM C:P in all biomes except KNZ, where C:P did not significantly differ between resources (Fig. 3.3E) Samples from LUQ exhibited the highest degree of C:P reduction (~13×) between CBOM and FBOM (Fig. 3.3E). Differences in N:P between CBOM and FBOM were only significant at LUQ and CWT (Fig. 3.2F), where CBOM N:P was 2-3× higher than FBOM N:P (Fig. 3.3F).

For ^{13}C, there was a significant interaction between sample type (CBOM, FBOM) and biome ($F_{3,196} = 121.28$, $p < 0.001$). Within each biome, CBOM was depleted in ^{13}C relative to FBOM (Fig. 3.4; CPC: $F_{1,32} = 56.19$, $p < 0.001$; CWT: $F_{1,73} = 33.95$, $p < 0.001$; KNZ: $F_{1,25} =$

128.57, $p < 0.001$; LUQ: $F_{1,66} = 147.48$, $p < 0.001$). There were significant differences in CBOM ^{13}C among biomes (Fig. 3.4A; $F_{3,29} = 31.48$, $p < 0.001$); LUQ CBOM was depleted in ^{13}C relative to the other three biomes, which were not significantly different from each other. There were also significant differences in FBOM ^{13}C among biomes (Fig. 3.4B; $F_{3,29} = 46.79$, $p < 0.001$); LUQ and CWT were depleted in ^{13}C relative to CPC and KNZ, while KNZ FBOM was significantly less depleted than CPC. There was also a significant sample by biome interaction for ^{15}N ($F_{3,196} = 2.89$, $p = 0.04$). Within each biome, CBOM was depleted in ^{15}N relative to FBOM (Fig. 3.4; CPC: $F_{1,32} = 93.04$, $p < 0.001$; CWT: $F_{1,73} = 662.24$, $p < 0.001$; KNZ: $F_{1,25} = 18.80$, $p < 0.001$; LUQ: $F_{1,66} = 277.23$, $p < 0.001$). There were significant differences among biomes for ^{15}N of both CBOM (Fig. 3.4A; $F_{3,29} = 38.27$, $p < 0.001$) and FBOM (Fig. 3.4B; $F_{3,29} = 50.86$, $p < 0.001$). For both resources, ^{15}N was most depleted at CWT, and least depleted at KNZ.

Variability in elemental mass and stoichiometry of CBOM and FBOM

The variability of nutrient content and stoichiometric ratios of basal resources was generally higher in CBOM than FBOM regardless of spatial extent considered (Fig. 3.5), as there were no significant interactions between resource and sampling extent. Variability was only higher in FBOM for % C ($F_{1,70} = 44.21$, $p < 0.001$). In contrast, CBOM was more variable than FBOM in % P ($F_{1,70} = 13.60$, $p < 0.001$), C:N ($F_{1,70} = 21.81$, $p < 0.001$), and C:P ($F_{1,70} = 10.94$, $p = 0.002$). The variability of the two resource pools was not different for % N ($F_{1,70} = 0.39$, $p = 0.53$) or N:P ($F_{1,70} = 0.99$, $p = 0.32$). Variability of resources generally did not change with the spatial extent of sampling (Fig. 3.5). Only C:N CV changed with sampling extent; variation was

the same within a biome as within a stream, but was significantly higher for both CBOM and FBOM when assessed across biomes ($F_{2,70} = 6.48$, $p = 0.003$).

Organic matter stoichiometry and consumer threshold elemental ratios

Based on measured CBOM C:N and C:P, nutrient (N and/or P) limitation may be widespread in aquatic detritivores that feed on CBOM (Fig. 3.6A). Across biomes, 90% of CBOM samples had C:N values that exceeded the estimated shredder $TER_{C:N}$ of 26.8 (Tant and others 2013). Within biomes, the proportion of CBOM samples that exceeded the $TER_{C:N}$ ranged from 70% in CPC to 100% in CWT. CBOM C:P was not as strongly skewed above the estimated $TER_{C:P}$ of 1992 (Tant et al. 2013), though 59% of samples across biomes had higher C:P than the estimated $TER_{C:P}$. Within biomes, the proportion of CBOM samples exceeding the $TER_{C:P}$ ranged from 0% in KNZ to 80% in CWT. This suggests that if the estimated TERs are generally representative of shredding macroinvertebrates that feed on CBOM, many food resources from streams across our sampled biomes would result in nutrient (N and/or P) limitation, with 58% of CBOM samples indicating co-limitation of both N and P (Fig. 3.6A).

In contrast, FBOM C:N and C:P values suggested that C, rather than N or P, limitation may be more likely for collector-gatherers that feed on FBOM (Fig. 3.6B). Among FBOM samples, only 3.9 % had C:N that exceeded the estimated collector-gatherer $TER_{C:N}$ of 25.6 (Tant et al. 2013), and all of those samples were from within the CWT network. No sampled FBOM had C:P above the estimated $TER_{C:P}$ of 1108 (Tant et al. 2013).

Discussion

Large-scale patterns in detrital stoichiometry

Among stream networks encompassing tropical forest (LUQ), temperate deciduous forest (CWT), tallgrass prairie (KNZ), and boreal forest (CPC), we found relatively consistent patterns in the nutrient content, stoichiometry, and isotopic signatures between CBOM and FBOM basal resource pools. FBOM was generally more nutrient-rich than CBOM (lower C:N, C:P ratios), with less variable stoichiometric ratios (lower CVs) and less depleted C and N isotopes. This consistent pattern across biomes emphasizes the role of in-stream homogenization of CBOM with variable composition into FBOM that is higher quality and less variable. This homogenization is the consequence of microbial colonization and macroinvertebrate breakdown, and suggests that stream microbes readily metabolize recalcitrant CBOM carbon (Marín-Spiotta et al. 2014). In contrast to the low variability of FBOM, CBOM across biomes was representative of the varying quality of allochthonous inputs among stream networks, which persisted despite fungal conditioning of litter inputs. The differences in isotopic signatures for ^{13}C between CBOM and FBOM across biomes also suggest that the source of FBOM may differ among stream networks. While CBOM ^{13}C was significantly depleted compared to FBOM in all four biomes, the difference from FBOM was most pronounced in CPC and KNZ, which suggests that FBOM in those biomes may contain more algal-sourced material than in LUQ and CWT, where the ^{13}C signatures suggest that FBOM originates from CBOM. However, previous research also indicates that microbial decomposition can contribute to significant enrichment of FBOM ^{13}C relative to CBOM (Finlay 2001). In addition, enrichment in the ^{15}N of FBOM across all four biomes suggests that microbial processing of CBOM is contributing to changes in the isotopic signatures of basal resources. Future comparisons that include algal, bacterial, and

fungal isotopic signatures across biomes could help resolve the degree to which observed differences in ^{13}C and ^{15}N were driven by differences in source material for CBOM and FBOM, versus microbial processing of CBOM to FBOM.

We observed some biome-specific differences in the nutrient content and stoichiometry of basal resources that affected the proportional change in stoichiometry between CBOM and FBOM. Both C:P and N:P changed significantly more between CBOM and FBOM in LUQ than in other biomes. This may reflect a legacy of terrestrial detrital quality that served as the source CBOM, as tropical foliage has high proportions of soluble P relative to soluble C and N (Schreeg et al. 2013). Leaching of P from litter before it enters streams as CBOM could contribute to higher C:P and N:P in LUQ CBOM. This is also consistent with previous findings of higher % N in tropical than temperate leaf litter (Ardón et al. 2009), and may reflect higher rates of biological nitrogen fixation in tropical forests (Cusack et al. 2009). Interestingly, the N:P of CBOM and FBOM did not significantly differ in either KNZ or CPC, and may reflect differences in nutrient limitation in these sites (Table 3.1; strong P-limitation in CPC, N-limitation in KNZ), and/or differences between streams in heavily forested biomes (LUQ, CWT) compared to biomes with a mixture of forest and herbaceous understory. Further investigations to pinpoint the mechanistic drivers of these cross-biome differences would help resolve this pattern.

While we detected differences in CBOM and FBOM elemental mass, stoichiometry, and isotopes across space, our one-time sampling represented a snapshot of a temporally variable resource base. Streams in temperate forests, such as those at CWT, experience annual pulses of organic matter input coincident with autumn leaf fall (Benfield et al. 2000), and conditioning by aquatic microbes changes the chemical composition of litter over time (Tant et al. 2015, Manning et al. 2016), and in turn, feeding by macroinvertebrates (Golladay et al. 1983). Our

current analysis assumed that the standing stocks of sampled resource pools were similar in each network with respect to the duration of conditioning, and some variability in resource stoichiometry across biomes could be due to differences in time since deposition in the stream. In general, resources were collected during summer, when litterfall and CBOM standing stocks in temperate streams tend to be low relative to peak inputs in late fall (Gray 1997, Benfield et al. 2000), and are expected to be low in boreal streams, which experience peak organic matter flushing during snowmelt (Petrone et al. 2006) and high CBOM standing stocks in early fall (Jones 1997). While Puerto Rico was sampled during winter, the collection still coincided with the months of annual litterfall minima (Zou et al. 1995).

Previous syntheses of terrestrial and lacustrine basal resources have found that despite some biome-specific differences, there are relatively narrow bounds on basal resource stoichiometry within ecosystem types (Elser et al. 2000, McGroddy et al. 2004, Cleveland and Liptzin 2007). In terrestrial ecosystems, litter C:N tends to be globally constant, despite biome-level differences in foliage stoichiometry, which reflects similar mechanisms of resorption across diverse species (McGroddy et al. 2004). Ratios of CBOM C:N in this study were more similar to previously compiled ratios of green foliage than terrestrial litter (foliage: 43.6 ± 3.5; litter: 66.2 ± 6.3), and mean C:P of CBOM fell between the values reported for foliage (1334 ± 138) and litter (3144 ± 342; McGroddy et al. 2004). This may be due to counteracting processes of resorption of foliar nutrients and microbial conditioning of CBOM. Resorption of foliar nutrients prior to leaf senescence results in significantly higher C:N and C:P in litter than foliage (McGroddy et al. 2004). In-stream colonization by fungi, and to a lesser extent, bacteria, on CBOM then decreases litter C:N and C:P, resulting in higher-quality FBOM (Findlay et al. 2002, Danger et al. 2016). In our study, FBOM was similar to C:N and C:P ratios previously reported for lake seston and soils

(Elser et al. 2000, Cleveland and Liptzin 2007), indicating that these composite pools of fine detritus and microorganisms are nutritionally similar across diverse ecosystem types.

Although N:P ratios did not significantly differ between the terrestrial and lake resources compiled by Elser and others (2000), N:P of CBOM in our streams was significantly higher than terrestrial samples, and N:P of FBOM was intermediate between lake seston and soils. This indicates that stream resources may tend to be enriched in N, relative to P, compared to patterns previously seen in lake and terrestrial data (Elser et al. 2000), and may be the result of low water column SRP availability in our streams relative to DIN, particularly in CPC (Table 3.1).

Implications for biogeochemistry

Understanding the magnitude and variability of basal resource stoichiometry can provide insights into carbon and nutrient cycling and retention in streams. An analysis across diverse ecosystems, including streams, found that nitrate availability is inversely correlated with organic carbon concentration, and that microbial stoichiometry and TERs can be used to predict nitrogen immobilization in microbial biomass versus nitrogen accrual in the environment (Taylor and Townsend 2010). A recent synthesis in aquatic ecosystems indicated that microbial stoichiometry, in conjunction with thermodynamic constraints, can be used to predict coupled cycling of C and N (Helton et al. 2015). In streams, detritus is high in C relative to N, leading to low rates of N turnover (Dodds et al. 2004). As N availability in streamwater increases, resource C:N is expected to decrease, and eventually reach a breakpoint beyond which biotic demand is fulfilled and N retention in streams decreases (Dodds et al. 2004, Taylor and Townsend 2010). This was shown experimentally when high C:N detritus was excluded from a stream, which resulted in increased downstream N export (Webster et al. 2000), and emphasizes the importance

of CBOM stoichiometry as a potentially important determinant of N retention in streams. Because headwater streams tend to be hotspots of nutrient retention (e.g., Peterson et al. 2001), understanding the magnitude and variability of resource stoichiometry in headwater networks from multiple biomes provides an important baseline for estimating the potential for nutrient uptake and retention in these systems. The C:N of CBOM was consistently higher than C:N of FBOM across all biomes studied here, suggesting there may be higher potential for N uptake by CBOM than FBOM. However, because we also found higher variability in CBOM than FBOM stoichiometric ratios, using CBOM C:N to estimate network-scale nitrogen retention could lead to under- or overestimation. Similarly, the P content of leaf litter has been shown to influence P retention in streams, with the highest P retention on low-P leaves (Aldridge et al. 2009). This suggests that CBOM, with its high C:P ratios, may retain more P in streams than lower C:P FBOM. Future studies that assess the mechanisms behind these relationships would prove useful in understanding how CBOM and FBOM stoichiometry could facilitate N and P retention in streams.

Consumer-resource elemental imbalances

Our estimates of consumer nutrient limitation are constrained by the availability of calculated C:N and C:P TERs, but our data on basal resource stoichiometry suggest that consumer-resource mismatches could be widespread across biomes, but differ between functional feeding groups. Shredding macroinvertebrates that consume CBOM may be consistently limited by N and/or P, based on stoichiometry of CBOM and estimated TERs. In contrast, collector-gatherers consuming FBOM may more often experience limitation by C than N or P. If this is the case, we would expect shredders to contribute to carbon use and cycling in

streams, while collector-gatherers would play a larger role in mobilizing nutrients in order to obtain sufficient carbon (Sterner and Elser 2002, Cross et al. 2005).

While herbivores and detritivores tend to have low growth efficiencies due to consumption of low-quality (high C:nutrient) foods (Elser et al. 2000), resources that are rich in nutrients relative to carbon may also reduce consumer growth (Boersma and Elser 2006). Biome-specific differences in resource quality could affect the degree of nutrient limitation experienced by local consumers, as aquatic detritivore phylogeny is predicted to be a stronger driver of body stoichiometry, and thus nutrient limitation, than the local environment (Cross et al. 2005 and references therein; Evans-White et al. 2005). Higher-quality (low C:nutrient) CBOM, as was collected from KNZ and CPC, may reduce nutrient limitation for shredding detritivores in those streams, which could result in increased growth rates and survival of those organisms (Danger et al. 2013) compared to shredders at LUQ and CWT. However, previous research has found that organisms experience unimodal growth responses to increasing food nutrient content (Boersma and Elser 2006 and references therein), which suggests that low C:P food resources could also reduce detritivore growth rates. This response to high-quality food is predicted to be particularly strong in detritivores that have evolved in chronically nutrient-stressed environments (Boersma and Elser 2006), which would include many undisturbed streams with low nutrient availability. While shredder responses to high-quality foods may be biome-specific, collector-gatherers, feeding on relatively high-quality FBOM, may experience C limitation rather than nutrient limitation across all sampled biomes. However, since available estimates of elemental imbalances are based on single field studies, specific organism TERs and thus the frequency of C or nutrient limitation is likely to vary. In addition, an important caveat to our findings is that bulk resources are not necessarily indicative of consumer feeding, as selective feeding by

macroinvertebrates may constrain the degree of nutrient limitation experienced compared to observed patterns of resource stoichiometry assessed from bulk samples (Hood et al. 2014). Despite these limitation, our preliminary estimates of detritivore carbon and nutrient limitation indicate that consumer-resource imbalances may be widespread, and that future studies that assess organism-specific gross growth efficiencies and assimilation efficiencies will facilitate more specific estimates of consumer C or nutrient limitations (Halvorson et al. 2015).

Potential changes to detrital resources with nutrient enrichment

Increased nitrogen and phosphorus availability in the water column tends to reduce the C:N and C:P ratios of CBOM and FBOM, and in turn alters the processes those resources mediate (Cross et al. 2003, Rosemond et al. 2010, Scott et al. 2013, Tant et al. 2013, Manning et al. 2015, Prater et al. 2015). Reduced CBOM C:N and C:P ratios accelerate breakdown rates through microbial, fungal and macroinvertebrate pathways, even at low-to-moderate nutrient concentrations (Kominoski et al. 2015, Manning et al. 2015). In addition, nutrient enrichment could affect the diversity of stream detritivores, as shredder and collector-gatherer richness tends to decrease with increasing streamwater phosphorus (Evans-White et al. 2009). Since our samples were collected from relatively undisturbed headwater networks within LTER sites, information on their nutrient content and stoichiometry can provide a baseline against which to compare resources under future scenarios of land use change and nutrient enrichment.

Our data provide a unique comparison of broad-scale variability in the stoichiometry of two basal resource pools, and potential implications of those ratios for consumers. This cross-biome assessment indicated that resource quality consistently increases between CBOM and FBOM across distinct biomes, via decreased C:N and C:P, and suggests that consumers of FBOM may broadly experience less nutrient (N and/or P) limitation than CBOM consumers.

Further assessments of the variability of detrital resource quality over seasons will further advance our cross-site understanding of ecological stoichiometry in headwater streams.

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Table 3.1. Physical and chemical characteristics of streams sampled for coarse (CBOM) and fine (FBOM) benthic organic matter stoichiometry. Network name indicates the Long Term Ecological Research (LTER) site affiliated with sampled streams. Sampling periods indicate the months during which samples were collected. Reaches (#) indicates the number of stream reaches sampled within each network. Discharge (Q) was measured in the field using a conservative tracer release, and elevation (Elev.) and upstream drainage area were quantified from a high-resolution digital elevation model. Dissolved inorganic nitrogen (DIN: nitrate [NO_3] and ammonium [NH_4]) and soluble reactive phosphorus (SRP) were measured at least once in each sampled reach. Values show the range encompassed by sampled streams, except DIN, SRP, and DIN:SRP, which report mean ± 1 SE, and DIN:SRP, which reports the dissolved molar N:P ratio based on mean DIN and SRP for each network.

Network	**Biome**	**Sampling period(s)**	**Reaches (#)**	**Q ($L\ s^{-1}$)**	**Elev. (m)**	**Upstream area (km^2)**	**DIN ($\mu g\ L^{-1}$)**	**SRP ($\mu g\ L^{-1}$)**	**DIN:SRP**
Luquillo (LUQ)	Tropical forest	Feb.-Mar. 2013, Mar. 2014	9	1.78-1472	77-673	0.09-17.80	91.9 ± 6.6	11.38 ± 0.80	19.9 ± 1.7
Coweeta (CWT)	Temperate deciduous forest	July-Aug. 2013	15	0.95-881	704-1025	0.09-15.32	71.6 ± 11.3	3.65 ± 0.36	63.0 ± 7.2
Konza (KNZ)	Tallgrass prairie	May-Jun. 2013	4	0.53-0.97	323-362	1.27-16.23	9.5 ± 0.9	7.51 ± 1.15	3.6 ± 0.6
Caribou Poker Creek (CPC)	Boreal forest	Jul.-Aug. 2013	8	16.4-451	218-330	5.96-60.00	342.6 ± 30.6	1.36 ± 0.23	591 ± 70

Table 3.2 Mean values ± 1 SE for coarse (CBOM) and fine (FBOM) benthic organic matter nutrient content (percent carbon [% C], nitrogen [% N], and phosphorus [% P]) and molar stoichiometric ratios (C:N, C:P, N:P) from sampled streams in four stream networks. Values in parentheses indicate range of each constituent. LUQ = Luquillo, CWT = Coweeta, KNZ = Konza, CPC = Caribou Poker Creek.

CBOM	**LUQ**	**CWT**	**KNZ**	**CPC**
% C	47.0 ± 0.4 (32.0 – 51.5)	47.2 ± 0.4 (39.3 – 50.6)	26.9 ± 2.5 (9.7 – 41.3)	33.8 ± 2.7 (12.8 – 49.4)
% N	1.36 ± 0.04 (0.78 – 2.01)	0.88 ± 0.04 (0.44 – 1.91)	1.03 ± 0.06 (0.68 – 1.37)	1.07 ± 0.04 (0.74 – 1.56)
% P	0.05 ± 0.003 (0.02 – 0.15)	0.05 ± 0.003 (0.02 – 0.10)	0.10 ± 0.01 (0.06 – 0.17)	0.07 ± 0.01 (0.03 – 0.13)
C:N	42.6 ± 1.3 (25.6 – 68.4)	68.6 ± 3.0 (27.9 – 121.6)	30.3 ± 2.2 (16.7 – 50.7)	36.3 ± 2.5 (16.5 – 53.9)
C:P	2657 ± 141 (843 – 5941)	3111 ± 203 (1228 – 7475)	721 ± 93 (246 – 1717)	1508 ± 237 (336 – 4603)
N:P	63.9 ± 3.5 (23.0 – 156.2)	44.4 ± 1.2 (27.2 – 61.5)	22.7 ± 1.5 (14.7 – 33.9)	37.7 ± 3.6 (20.1 – 85.4)
FBOM	**LUQ**	**CWT**	**KNZ**	**CPC**
% C	5.5 ± 0.3 (1.3 – 10.4)	10.1 ± 0.7 (2.7 – 20.9)	5.6 ± 0.6 (3.2 – 9.6)	4.0 ± 0.4 (2.5 – 9.1)
% N	0.57 ± 0.02 (0.22 – 0.85)	0.52 ± 0.03 (0.20 – 1.13)	0.54 ± 0.02 (0.40 – 0.64)	0.48 ± 0.02 (0.40 – 0.75)
% P	0.07 ± 0.002 (0.03 – 0.09)	0.05 ± 0.003 (0.02 – 0.11)	0.05 ± 0.003 (0.03 – 0.07)	0.03 ± 0.002 (0.02 – 0.05)
C:N	11.06 ± 0.27 (4.4 – 16.1)	22.36 ± 0.37 (15.0 – 26.3)	12.27 ± 1.34 (7.4 – 23.0)	9.16 ± 0.49 (6.9 – 15.2)
C:P	223 ± 10 (45 – 370)	493 ± 29 (263 – 1103)	288 ± 33 (171 – 627)	336 ± 35 (157 – 1010)
N:P	20.1 ± 0.8 (6.4 – 34.7)	22.0 ± 1.3 (12.3 – 58.1)	23.7 ± 1.0 (18.1 – 32.3)	35.4 ± 2.2 (22.6 – 71.6)

Figure Legends

Fig. 3.1 Sampled headwater stream networks in four biomes: boreal forest (Caribou Poker Creek; CPC), temperate tallgrass prairie (Konza Prairie; KNZ), temperate deciduous forest (Coweeta; CWT) and tropical rainforest (Luquillo; LUQ). Points indicate sites of basal resources collection within each network. Map of Puerto Rico is enlarged relative to contiguous United States and Alaska to show detail of network location. Note that scale bars vary by network. See text and Table 3.1 for site descriptions.

Fig. 3.2 Percent elemental mass (A: percent carbon [% C], B: nitrogen [% N], and C: phosphorus [% P]) and stoichiometric molar ratios (D: carbon:nitrogen [C:N], E: carbon:phosphorus [C:P], F: nitrogen:phosphorus [N:P]) for coarse (CBOM; gray) and fine (FBOM; white) benthic organic matter in streams of stream networks from four biomes (LUQ, CWT, KNZ, CPC). ALL represents samples pooled across biomes. Boxes indicated 25^{th} and 75^{th} percentile while whiskers indicate 10^{th} and 90^{th} percentile. Asterisks indicate significant differences between CBOM and FBOM (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$); NS = not significant.

Fig. 3.3 Proportional change in nutrient content (A: percent carbon [% C], B: nitrogen [% N], and C: phosphorus [% P]) and stoichiometric molar ratios (D: carbon:nitrogen [C:N], E: carbon:phosphorus [C:P], F: nitrogen:phosphorus [N:P]) between CBOM and FBOM within four sampled biomes. Values represent CBOM / FBOM for each nutrient or ratio; small dots within a biome indicate stream means, large dots indicate biome mean. Values above the dashed line (=1) indicate higher nutrient content or ratios in CBOM than FBOM; values below the dashed line

indicate higher content or ratio in FBOM. Letters indicate significant differences ($p < 0.05$) between biomes based on pairwise comparisons of least-squares means.

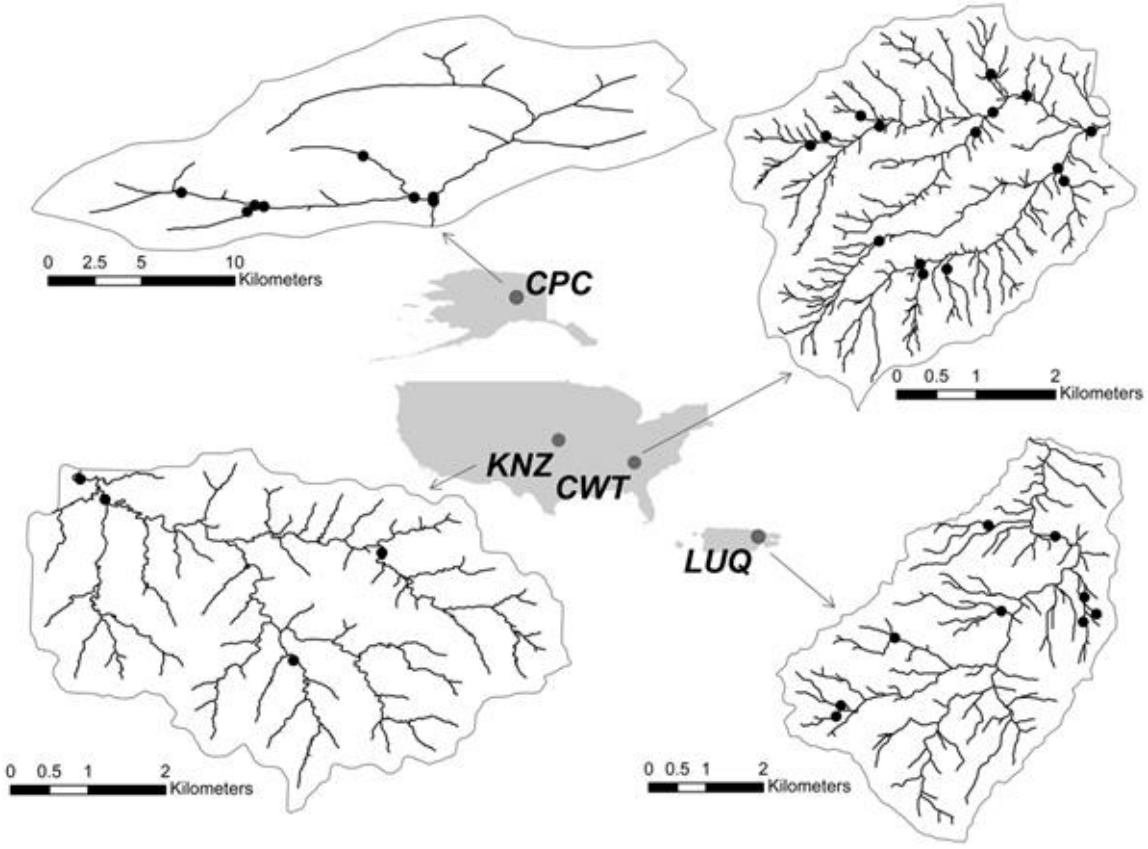
Fig. 3.4 Delta ^{15}N ($\delta^{15}N$) as a function of delta ^{13}C ($\delta^{13}C$) for (A) coarse and (B) fine benthic organic matter in four headwater stream networks (LUQ: light gray circle; CWT: black square; KNZ: dark gray triangle; CPC: white upside down triangle). Ellipses indicate 95% confidence levels of sample points for each biome (LUQ: light gray; CWT: black; KNZ: dark gray; CPC: dotted black).

Fig. 3.5 Coefficient of variation for percent elemental mass (% C, % N, % P) and molar ratios (C:N, C:P, and N:P) of (A) coarse and (B) fine benthic organic matter sampled from stream networks in four biomes. Within a stream represents the mean (± 1 SE) CV of the carbon pool sampled from multiple replicates within a single stream reach in each network (n=36). Within a network represents the mean (± 1 SE) CV for each detrital pool across multiple streams within a given network (n = 4). Across biomes is the mean CV of all samples collected across the four networks (n = 1).

Fig. 3.6 Carbon:phosphorus (C:P) ratios as a function of carbon:nitrogen (C:N) ratios for (A) coarse and (B) fine benthic organic matter in headwater stream networks in four biomes (LUQ: light gray circle; CWT: black square; KNZ: dark gray triangle; CPC: white upside down triangle). Ellipses indicate 95% confidence levels of sample points for each biome (LUQ: light gray; CWT: black; KNZ: dark gray; CPC: dotted black). Thin dotted black lines correspond to mean threshold elemental ratios (TERs) of C:N (vertical) and C:P (horizontal) for

macroinvertebrate (A) CBOM shredders and (B) FBOM collector-gatherers. Mean TERs are from Tant and others (2013), based on body stoichiometry from Cross et al. (2003). Gray shaded areas correspond to TERs estimated as ± 1 SE of body stoichiometry from Cross et al. (2003). Values below (C:P) or to the left (C:N) of the TER line indicate areas of potential consumer carbon limitation, while values above (C:P) and to the right (C:N) of the TER line indicate areas of P and N limitation, respectively. Note that axis limits differ between (A) and (B).

Fig. 3.1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Kilometers
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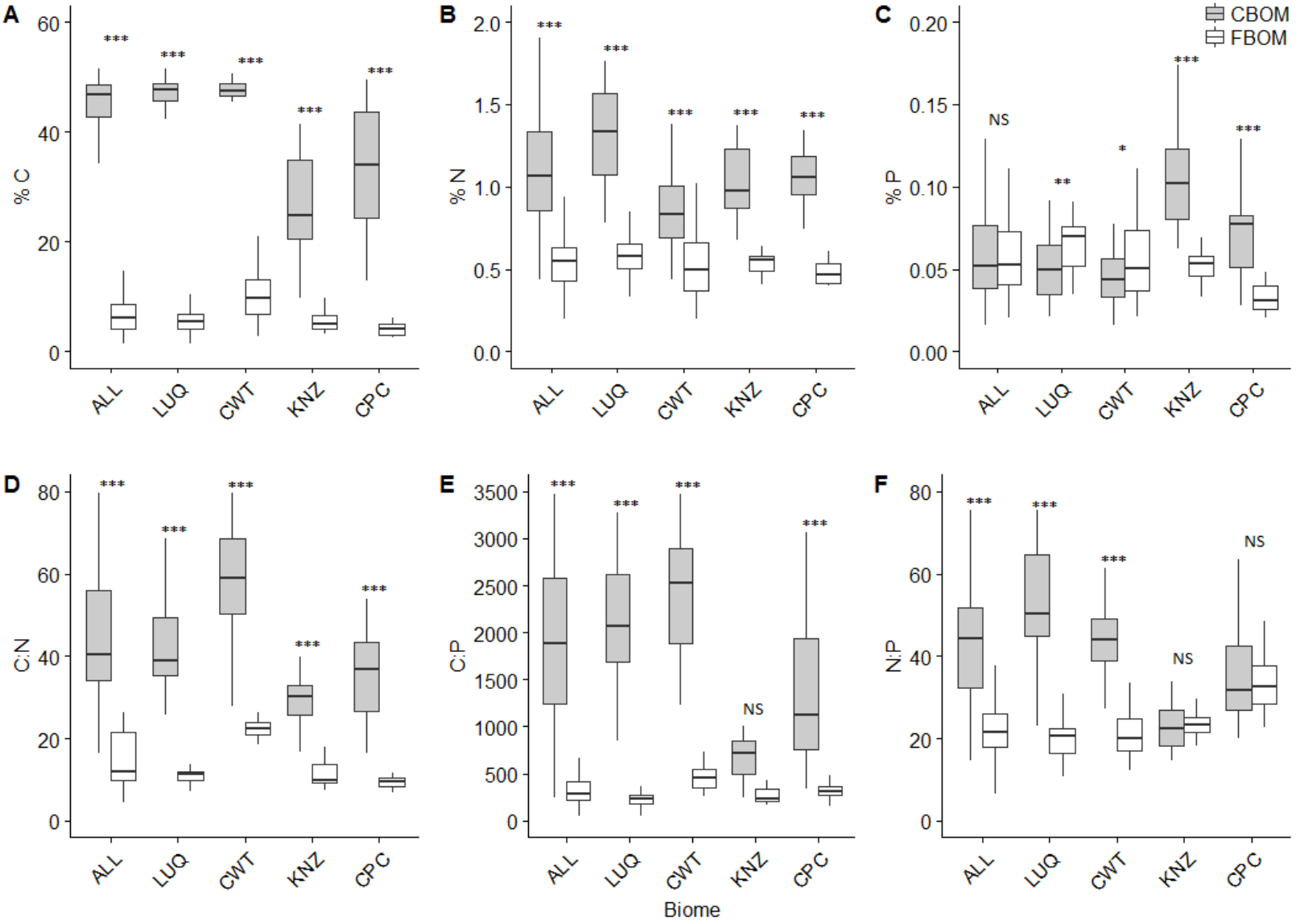
Fig. 3.2



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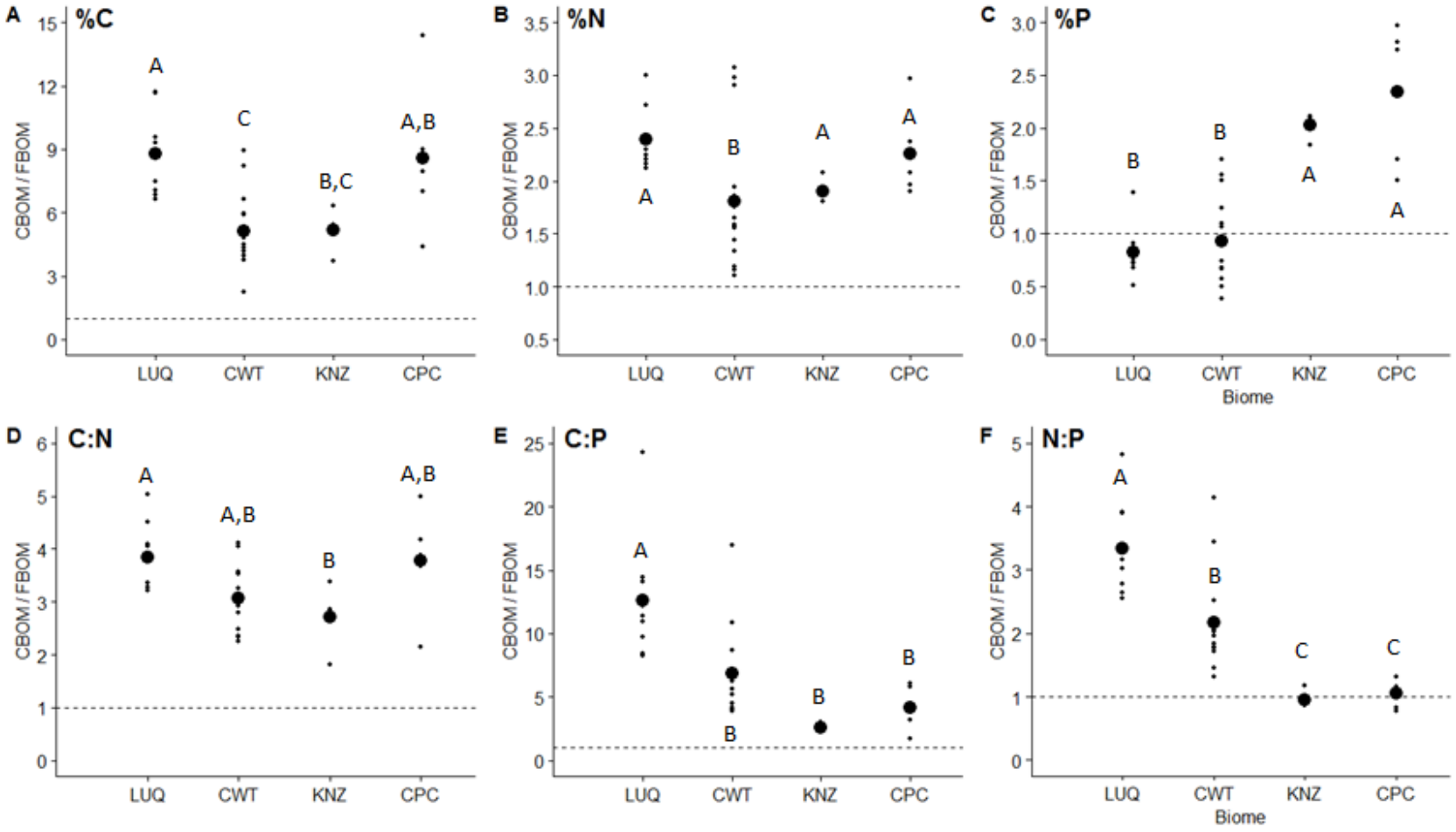
A
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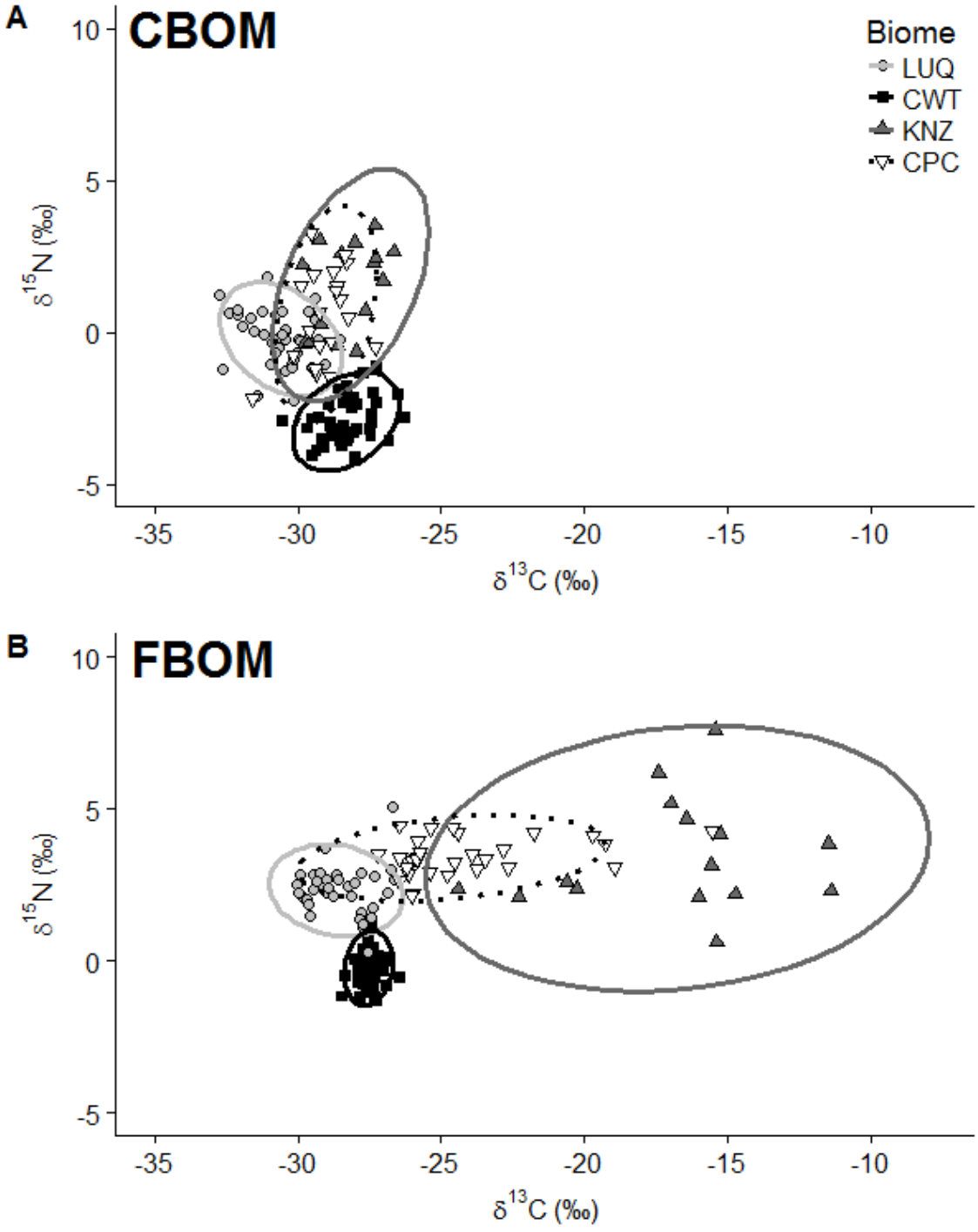
Fig. 3.4


A
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δ13C (‰)
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FBOM
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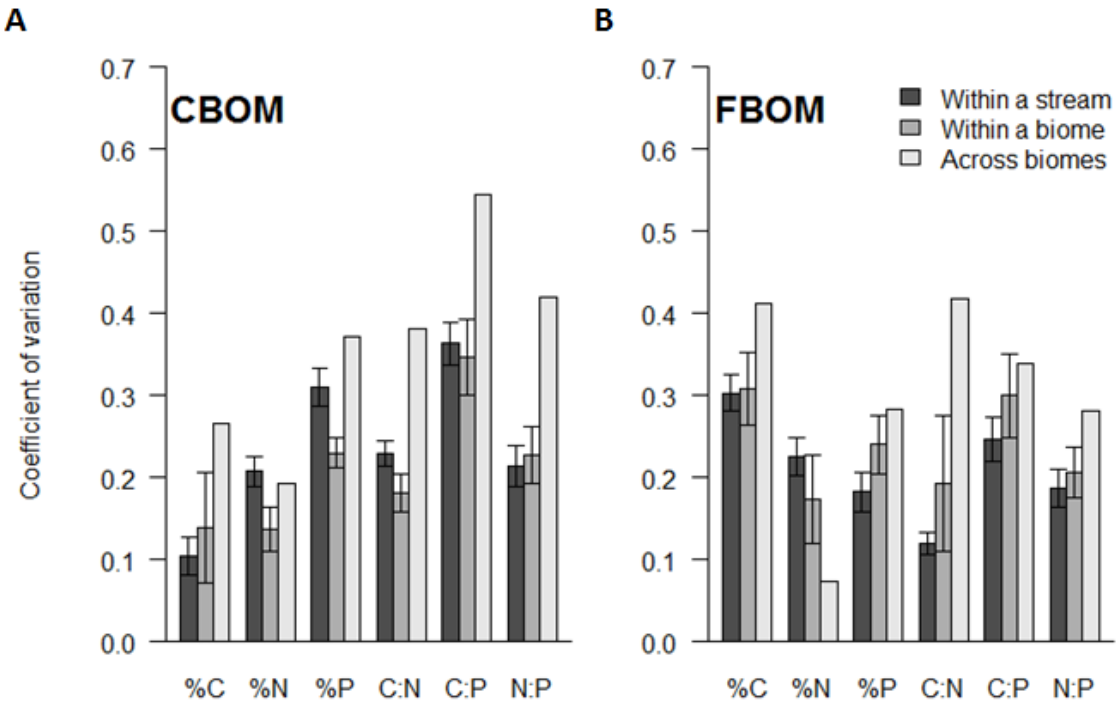
-10
δ13C (‰)

Fig. 3.5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CBOM
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Coefficient of variation
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0.1
0.2
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0.4
0.5
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%C
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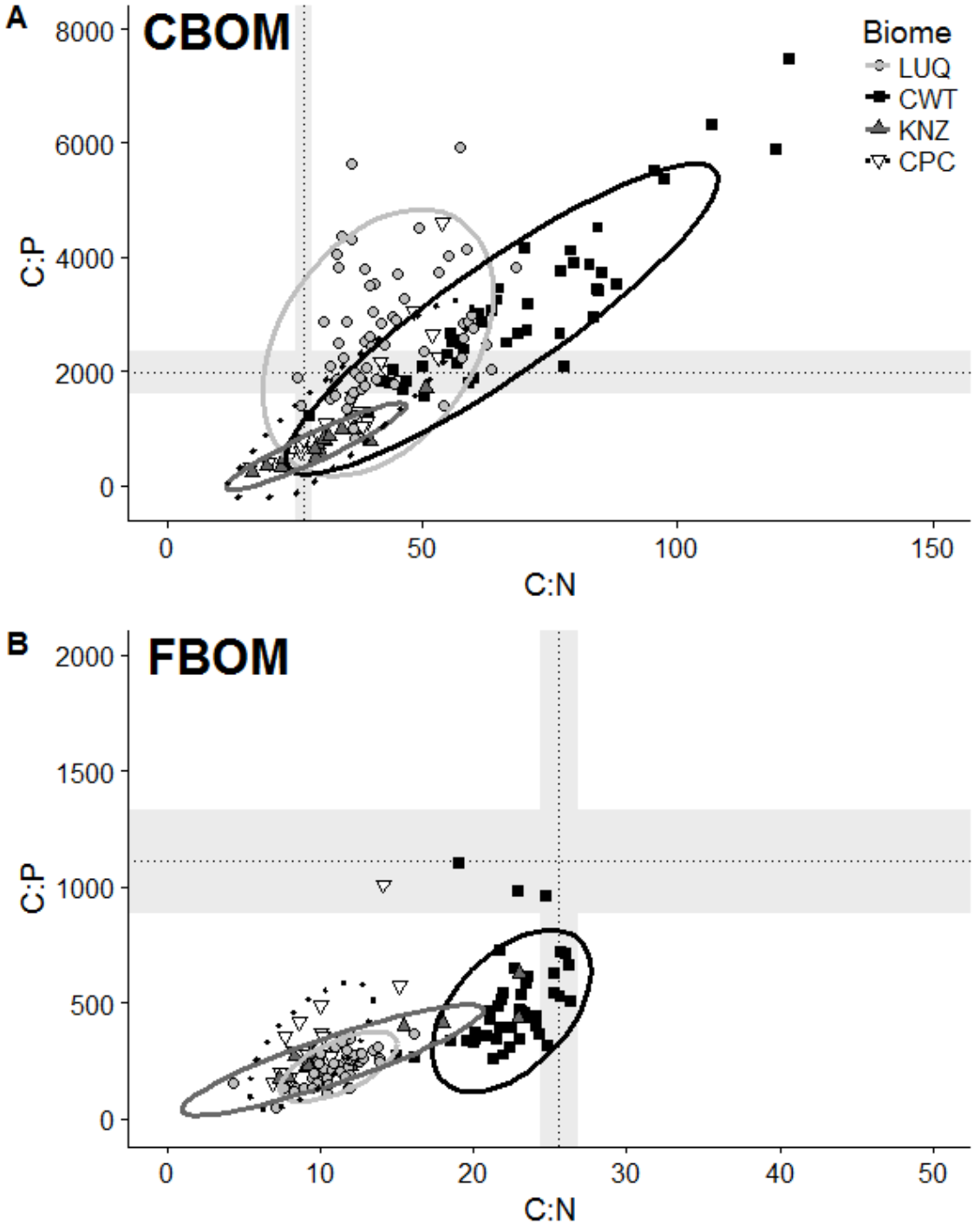
%P
C:N
C:P
N:P
Within a stream
Within a biome
Across biomes

Fig. 3.6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CHAPTER 4

EFFECTS OF ALTERED LARVAL SALAMANDER BIOMASS ON ECOSYSTEM STRUCTURE AND FUNCTION IN A HEADWATER STREAM[3]

[3] Farrell, K.J., A.D. Rosemond, P.M. Bumpers, and J.C. Maerz. To be submitted to *Hydrobiologia*.

Abstract

Aquatic consumers can shape communities and ecosystems through both top-down (predation) and bottom-up (nutrient cycling) pathways. Larval salamanders are abundant predators in southeastern streams and have been shown to reduce macroinvertebrate biomass, which could affect detrital standing stocks and loss rates. Salamanders may also alter stream nitrogen cycling, through storage of nutrients in body tissues and release of nitrogen through excretion. While results of previous experiments are equivocal, understanding salamander effects is important given ongoing threats to their habitats via land use and climate changes. Here, we used an enclosure experiment to measure effects of larval *Desmognathus quadramaculatus* (Dq) salamanders on ecosystem processes associated with detrital resources in a southeastern headwater stream. We quantified macroinvertebrate biomass and community structure, and coarse (CBOM) and fine (FBOM) benthic organic matter standing stocks across a gradient of salamander biomass, and measured ecosystem functions using leaf packs (leaf breakdown rate [*k*]) and recirculating chambers (respiration, ammonium [NH_4] uptake). Increased salamander biomass did not reduce macroinvertebrate biomass or change functional feeding group (FFG) composition. Across all salamander treatments, shredders were the dominant FFG by biomass, while collector-gatherers had the highest abundance. Contrary to our expectations, macroinvertebrate shredder biomass was not correlated with CBOM standing stock or *k*. Measured NH_4 uptake rates were inversely related to salamander biomass. However, our other measured ecosystem functions (*k*, respiration) were not related to salamander biomass. While we did not detect top-down salamander effects on macroinvertebrate biomass, nor cascading effects to CBOM or FBOM standing stocks or respiration rates, our results highlight the potential importance of salamander excretion on stream nutrient dynamics. Evidence for biotic control

was relatively weak in our study, but limitations in inference from short-term field manipulations should also be considered.

Introduction

Tests of the extent to which top-down (consumer) versus bottom-up (resource) drivers structure communities in freshwater ecosystems are widespread in the literature. The net effects of herbivores and predators can include reductions of prey via consumption, but also stimulation of primary producers through the excretion of limiting nutrients (Vanni 2002). In lakes, zooplankton herbivory reduces phytoplankton abundance, while excretion stimulates phytoplankton growth, and the relative contribution of top-down and bottom-up control of systems can shift seasonally (Vanni & Temte, 1990). Similar effects of herbivores have been observed in streams, where aggregations of algivorous fishes can simultaneously reduce algal standing stocks via consumption while stimulating primary productivity through nitrogen excretion (Capps et al. 2014). While these cases both tested two-level trophic interactions (herbivore to primary producer), net effects of predators in systems with longer food chain lengths may be more nuanced, as higher-level consumers can indirectly relieve predation pressure on primary consumers and/or basal resources as well as contribute to nutrient cycling (McQueen et al. 1989). In streams and rivers, experiments testing the existence and importance of top-down control have often examined the role of predatory fishes in reducing macroinvertebrate biomass (e.g., Allan 1982, Flecker 1984, Gibson et al. 2004), and whether macroinvertebrate reductions propagate to affect basal resources including algal and detrital standing stocks (Power 1990, Rosemond et al. 2001, Ruetz et al. 2002, 2004). However, the observed strength of top-down control is variable (Wooster 1994), and appears to depend on

predator feeding mode (Flecker 1984, Dahl and Greenberg 1996, Cheever and Simon 2009), habitat complexity (Dahl and Greenberg 1996), experimental timing (Cheever and Simon 2009), the presence of other invertebrate or vertebrate consumers (Dahl and Greenberg 1996, Gibson et al. 2004), or a combination of these factors.

Despite conflicting evidence as to the relative importance of top-down and bottom-up macroconsumer control in stream ecosystems, there is widespread concern as to how the loss of consumers will affect ecosystems (Estes et al. 2011, Dirzo et al. 2014, Young et al. 2016). The ongoing 6th mass extinction event (Ceballos et al. 2015) appears to be affecting large-bodied and apex consumers disproportionately (Dirzo et al. 2014, He et al. 2017), leading to 'trophic downgrading' of ecosystems worldwide (Estes et al. 2011). While responses to consumer loss may take years to decades to become fully evident in natural systems (Estes et al. 2011), understanding potential outcomes of consumer loss can be preliminarily examined by experimentally manipulating the density and biomass of consumers.

In fishless headwater streams, larval salamanders can serve as a dominant vertebrate predator, and are hypothesized to act as regulators of both macroinvertebrate communities and nutrient cycling rates (Davic and Welsh 2004). The Southern Appalachian Mountains are a global hotspot for salamander species richness, and fishless headwater streams in the region often include 3-5 plethodontid salamander taxa including *Desmognathus*, *Eurycea*, *Gyrinophilus*, and *Pseudotriton* species (Petranka 1998, Davic and Welsh 2004). Numerous studies have quantified the effects of plethodontid salamander predation on macroinvertebrate biomass and abundance in streams (e.g., Davic 1983, Keitzer and Goforth 2013a), but evidence to date indicates that top-down effects of salamanders on macroinvertebrate communities, and on macroinvertebrate-mediated litter breakdown rates, are equivocal. Experimental reductions of

salamander abundance in headwater streams have been shown to both increase and decrease macroinvertebrate abundance, with the detection of top-down effects dependent upon both stream reach and season (Davic 1983). While salamanders significantly slowed litter breakdown rates by reducing the density of shredding macroinvertebrates in one experimental reach, this result depended on sampling season and was not detected in a replicate reach (Davic 1983). Similarly, in an enclosure experiment, significant reductions in macroinvertebrate abundance were dependent upon salamander community composition, and disproportionately affected a single macroinvertebrate taxon (Keitzer and Goforth 2013a), with unknown implications for stream functions, including litter breakdown.

For salamander-driven changes in macroinvertebrates to cascade to quantifiable differences in detrital standing stocks and rates of litter breakdown or ecosystem respiration, salamander predation must substantially decrease the abundance of shredding macroinvertebrates. Studies of larval *Desmognathus quadramaculatus* diets have estimated that shredders typically constitute 26 – 44% of prey (Davic 1983, Bumpers 2014), so top-down effects on ecosystem processes would likely be subtle. However, larval salamanders can also alter stream nutrient cycling through both storage of limiting nutrients (e.g., nitrogen, phosphorus) in body tissues and excretion of those nutrients (Davic and Welsh 2004, Keitzer and Goforth 2013b, Munshaw et al. 2013, Milanovich et al. 2015), though direct tests of the relationship between salamander biomass and nutrient uptake by the stream benthos are lacking. A more nuanced understanding of how salamanders may alter functions in streams via changes in macroinvertebrates and litter standing stocks is essential for predicting ecosystem-level consequences of salamander population declines under future climate and land use scenarios (Davic and Welsh 2004, Milanovich et al. 2010).

Here, we used an enclosure experiment to test the effects of a gradient of salamander biomass on litter breakdown, ecosystem respiration, and ammonium (NH_4) uptake via changes in macroinvertebrate biomass and organic matter standing stocks (Fig. 4.1). Specifically, we sought to quantify the net effects of top-down predation and bottom-up nutrient excretion by testing 1) whether salamander biomass was positively correlated with organic matter standing stocks via consumptive reductions of macroinvertebrate shredders, 2) whether salamander-mediated changes in organic matter standing stocks would cascade to detectable changes in ecosystem functions (decreased litter breakdown, increased respiration), and 3) whether salamander biomass was negatively correlated with NH_4 uptake rates (Fig. 4.1). To achieve these objectives, we deployed enclosures in a small 2nd order stream in the Southern Appalachian Mountains that were stocked with a gradient of salamander biomass. We hypothesized that as generalist insectivores (Bumpers 2014, Trice et al. 2015), salamanders would not alter macroinvertebrate community structure, but expected that they would reduce macroinvertebrate biomass and abundance, and thus, the abundance of shredders. We predicted that if increased salamander biomass reduced shredder biomass, there would be consequent reductions in leaf litter breakdown rates. We also expected that respiration and NH_4 uptake rates would be positively correlated with standing stocks of coarse benthic organic matter (CBOM), so if salamanders reduced shredder biomass, higher standing stocks of CBOM would increase measured respiration and NH_4 uptake (Fig. 4.1). Finally, we predicted that increased salamander biomass would correspond to reduced NH_4 uptake rates, via NH_4 excretion fulfilling ecosystem nitrogen demand.

Methods

Experimental enclosures

We conducted our experiment in Lick Branch (Watershed 22), a 2nd order stream within the US Forest Service Coweeta Hydrologic Laboratory and Long Term Ecological Research (LTER) site in Macon County, North Carolina, USA. The study stream drains 34 ha, with a riparian area dominated by a mixed hardwood overstory (primarily oak [*Quercus* spp.], tulip poplar [*Liriodendron tulipifera*], and red maple [*Acer rubrum*]), and a dense understory of *Rhododendron maximum* that shades the stream year-round (Swank and Crossley 1988).

We focused our salamander manipulation on larval blackbelly salamanders (*Desmognathus quadramaculatus* Holbrook, hereafter Dq), which are present in fishless headwater streams in Coweeta in high densities (15 – 32 individuals m^{-2}; Keitzer and Goforth 2012, Milanovich et al. 2015) and constitute the majority of larval salamander biomass (>90%; Milanovich et al. 2015) in a mixed community of up to seven salamander species in these streams. A previous assessment of Dq in our study stream estimated a mean of 32 individuals m^{-2}, though the 95% confidence interval in the stream ranged from 5 to 156 individuals m^{-2} (Milanovich et al. 2015). While larval *Eurycea wilderae* Dunn (Ew) have a higher abundance in Lick Branch (mean: 52 individuals m^{-2}, 95% confidence interval 2 – 457 individuals m^{-2}; Milanovich et al. 2015), they have a smaller mean body size, and contribute only ~7% to the larval community biomass in the stream (Milanovich et al. 2015). In addition, our experiment was initiated soon after the end of the larval hatching season for Ew (Bruce 1988), and captured individuals were too small to be reliably contained in our experimental enclosures (P. Bumpers, pers. observation).

We conducted an enclosure study to assess potential consequences of reduced Dq biomass on metrics of ecosystem structure and function. We installed plastic-framed mesh-sided enclosures (~0.52 m^2) in the stream such that each enclosure was flush with the streambed. We covered the bottom of each enclosure with a layer of cobbles and gravel to mimic the ambient stream substrates, and added 5.1 ± 0.1 g of air-dried mixed leaf detritus to mirror ambient benthic organic matter standing stocks. The mesh size of the enclosures (~1.5 × 1.5 mm) allowed water and macroinvertebrates to pass into and out of the enclosures, but retained Dq. Enclosures were installed in the stream on August 13, 2014, prior to stocking with salamanders on August 19, to allow for initial colonization of enclosures by macroinvertebrates.

To establish a gradient of Dq abundance and biomass, we randomly assigned 3 enclosures to each of 5 treatments (0, 3, 5, 10, or 15 Dq individuals per enclosure; n = 15 total enclosures) to achieve a gradient of 0 to ~30 individuals m^{-2} in our enclosures. Stocked salamanders were captured from Lick Branch or nearby, 1st order streams within the Coweeta LTER. The snout vent length (SVL: from the tip of the snout to the posterior portion of the vent) of each salamander was measured to the nearest 0.5 mm prior to placement in an enclosure, and biomass per enclosure was estimated based on a previously established relationship between SVL (mm) and wet mass (g) for Dq in Coweeta streams ($\ln(mass) = 2.97 \times \ln(SVL) - 10.51$, $R^2 = 0.87$; P. Bumpers, unpublished data). Salamanders were assigned to enclosures to create a similar distribution of size classes in each enclosure.

Leaf breakdown experiment

Leaf packs were assembled from senescent red maple (*Acer rubrum*) leaves collected previously in Coweeta. Leaves were air dried for a minimum of 3 weeks, then weighed into

5.200 ± 0.200 g leaf packs. Leaf packs were enclosed in 5 mm plastic mesh bags (Cady Bag Inc., Pearson, Georgia, USA) to allow access by macroinvertebrates and salamanders. Leaf packs were installed in enclosures on August 13, 2014. Ten packs were submerged in each enclosure, and each pack was secured in place on the enclosure bottom using a large cobble. A total of 150 packs (15 enclosures × 5 sampling dates × 2 replicates) were deployed. Two additional leaf packs were brought to the field, installed in an enclosure, and immediately collected to account for handling loss (Benfield 2006).

A subset of incubated litter bags was removed from each enclosure on the day salamanders were added to enclosures (day 7), and approximately every two weeks thereafter (day 14, 21, 34, 48, and 63). On each sampling day, two litter bags were randomly removed from each enclosure, placed into individual plastic bags, and transported on ice to the laboratory. In the lab, leaf litter was rinsed over nested 1 mm and 250 μm sieves to remove sediments and macroinvertebrates. Rinsed leaf material was placed into individually labeled paper bags and dried at 50°C for 72 hours. Dried leaf material from each pack was weighed to the nearest 0.001 g, and a subset of leaf material was combusted at 500°C for 4.5 hours, then cooled and reweighed to calculate relationships between dry mass and ash free dry mass (AFDM). We estimated leaf breakdown rate, *k*, using a linearized version of the negative exponential model from Benfield (2006): $\ln(M_t/M_0) = k \times t$, where M_t is the mass (g) of leaves remaining at time *t* (days), and M_0 is the initial leaf mass. We estimated separate *k* values for each enclosure.

Macroinvertebrates from each leaf pack were preserved in 70% ethanol, then sorted and identified to the lowest taxonomic level (typically genus; Merritt et al. 2008) and corresponding functional feeding group. Chironomidae were classified as either Tanypodinae or non-Tanypodinae. Macroinvertebrates were measured to the nearest millimeter, and biomass (mg dry

mass) was estimated from body length using previously published length-mass regressions for southeastern taxa (Benke et al. 1999). We focused macroinvertebrate identification on samples from day 63, to test whether there were differences in macroinvertebrate biomass and community composition at the end of the enclosure experiment.

Ecosystem rate measurements

Ecosystem rates (respiration, NH_4 uptake) were quantified using sets of 0.01 m^2 plastic mesh baskets filled with ambient stream substrates (mixed gravels and cobbles). Three baskets were installed in each enclosure on August 13. Eight weeks after salamanders were stocked in enclosures (October 15), we measured respiration and NH_4 uptake by running incubated baskets in recirculating 10 L chambers using methods detailed in Rüegg et al. (2015). Briefly, the set of 3 baskets was removed from an enclosure and placed in a chamber, with 5 chambers run simultaneously on the bank adjacent to the study stream. During chamber runs, dissolved oxygen and water temperature were logged at 1-minute intervals using a YSI ProODO sensor (Yellow Springs, Ohio, USA). Ecosystem respiration was quantified by covering chambers to block incident light (30 minutes). Following respiration measurements, DO sensors were removed, and a 3 mL slug of 0.46 g L^{-1} NH_4Cl solution was added to each chamber to achieve a target chamber-water concentration of 50 μg L^{-1} NH_4-N. Water samples were collected from each chamber 1, 3, 6, 12, 24, and 40- minutes post-slug addition to quantify NH_4 concentration. Water samples were filtered on site through a 0.7 μm glass fiber filter (GF/F; Sterlitech Corporation, Kent, Washington, USA), cooled on ice, and frozen (-20°C) upon return to the laboratory. In the laboratory, samples were defrosted and immediately analyzed for NH_4-N using the colorimetric

phenol-hypochlorite method (APHA 1995) and read on a spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) at 640 μm wavelength.

We calculated respiration based on the linear change in dissolved oxygen concentration in each chamber over time: $\text{respiration} = \left(\frac{[O_{2\,t}]-[O_{2\,t0}]}{t}\right) \times \left(\frac{V}{A}\right)$, where t is the elapsed time (min.), $O_{2\,t0}$ is the initial dissolved oxygen concentration in the chamber (mg L^{-1}), V is the chamber volume (L), and A is the area of the set of 3 baskets (0.03 m^2). Chamber NH_4 uptake was calculated as the linear change in the natural log of NH_4 concentration over time: $uptake = \left(\frac{ln[NH_{4\,t}]-ln[NH_{4\,t0}]}{t}\right) \times \left(\frac{V}{A}\right) \times NH_{4\,amb}$, where $NH_{4\,t0}$ and $NH_{4\,t}$ are the measured NH_4 concentrations (μg L^{-1}) of two water samples, $NH_{4\,amb}$ is the ambient streamwater NH_4 concentration (10 μg L^{-1}), and t is the elapsed time between water samples (min.).

We quantified coarse (CBOM) and fine (FBOM) benthic organic matter from baskets in each chamber as potential drivers of measured ecosystem rates. CBOM and FBOM were collected by pouring the contents of each chamber through a 1 mm sieve into a bucket. Detritus captured on the sieve was placed into a polyethylene bag as CBOM, while FBOM was collected by agitating the contents of the bucket and collecting a 250 mL subsample in a polyethylene bottle. All CBOM and FBOM samples were transported to the laboratory on ice.

In the lab, CBOM was quantified by placing collected detritus in individual pre-weighed aluminum tins and drying at 50°C for at least 72 hours. Samples were then weighed, ashed at 500°C for 4.5 hours, and reweighed for AFDM. The FBOM slurry from each chamber was resuspended, then filtered through a pre-weighed 0.7 μm GF/F until it clogged. Filtered volume was recorded and filters were dried, weighed, and ashed as described for CBOM.

Statistical analyses

We compared macroinvertebrate community composition among salamander treatments using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity index in the 'vegan' package in R (Oksanen et al. 2017). For each enclosure, we summed macroinvertebrate biomass by taxonomic group (genus for Insecta, otherwise order) from the two day-63 leaf packs, and built our community biomass matrix based on the summed values. We tested for significant differences between macroinvertebrate communities by salamander treatment using a permutational MANOVA based on the distance matrices (function 'adonis2'). All macroinvertebrate biomass values (mg DM) were natural log + 1 transformed prior to analysis to meet assumptions of normality.

Because of their predicted effects on litter breakdown and respiration (Fig. 4.1), we tested whether mean macroinvertebrate collector-gatherer and shredder biomass in an enclosure were related to standing stocks of organic matter using simple linear regression. Specifically, we tested whether mean collector-gatherer biomass was related to FBOM standing stock, and whether mean shredder biomass predicted either CBOM standing stock or *k*. We also tested whether predatory macroinvertebrate biomass was related to the biomass of non-predatory macroinvertebrates using simple linear regression.

We assessed whether macroinvertebrate total biomass or abundance was negatively correlated with salamander biomass using a linear mixed effects model ('nlme' package; Pinheiro et al., 2016) with a fixed effect of salamander biomass and a random effect of enclosure to account for pseudoreplicated macroinvertebrate samples (2 leaf packs per enclosure on day 63). We also used linear mixed effects models to test whether the biomass or abundance of each functional feeding group (FFG) changed as a function of a fixed effect of salamander biomass

and a random effect of enclosure. Macroinvertebrate data were normalized as mass (mg) or abundance (individuals) per gram of leaf pack AFDM. Salamander biomass was natural log + 1 transformed to account for 0 values from control enclosures, and macroinvertebrate data were natural log transformed to meet the assumptions of normality. Post hoc comparisons between functional feeding groups were conducted based on least-square means using the 'lsmeans' package (Lenth 2016).

To calculate leaf breakdown rate (*k*) per enclosure and assess effects of salamander abundance on *k*, we used a linear mixed effects model of the natural log of leaf pack AFDM remaining with an interaction between fixed effects of salamander abundance and days in the stream, and a random slope of enclosure nested within days-in-stream to account for two leaf packs being collected for each date/enclosure combination. The unique slope for each enclosure was the breakdown rate (*k*).

To assess whether salamander biomass and basal resource pools were significant predictors of ecosystem rates (respiration, NH_4 uptake), we developed candidate linear models for each rate. Candidate models included additive effects of proximate basal resource drivers and salamander biomass (Fig. 4.1), and we simplified models using a stepwise approach to remove terms that were not significant. The simplest candidate model for each rate did not include any drivers (i.e., intercept only). We used the AICcmodavg package (Mazerolle 2016) to rank candidate models using AIC_c to account for small sample sizes (Burnham and Anderson 2002). For both respiration and NH_4 uptake, hypothesized drivers included salamander biomass, CBOM and FBOM AFDM, and water temperature. For all models, drivers were natural log transformed (salamander biomass: natural log + 1) to meet assumptions of normality. All analyses were conducted using R 3.3.3 (R Core Team 2017).

Results

Effects on structure

Across all day-63 leaf packs, we identified 1024 macroinvertebrates from 11 orders, including 29 insect families. Salamander biomass did not significantly affect total macroinvertebrate biomass ($F_{1,13} = 0.28$, $p = 0.61$) or abundance ($F_{1,13} = 0.31$, $p = 0.59$), and macroinvertebrate community composition was not different among salamander treatments (Fig. 4.2A; $F_{4,10} = 1.13$, $p = 0.30$). However, there were significant overall differences between FFGs in both absolute biomass ($F_{4,71} = 3.30$, $p = 0.02$) and abundance (Fig. 4.2B-C; $F_{4,71} = 25.00$, $p < 0.001$). Shredders were the dominant FFG by biomass, and comprised 44 – 73% of collected macroinvertebrates in each leaf pack (Fig. 4.2B, Appendix B). Neither the biomass nor abundance of shredders differed with salamander enclosure biomass (biomass: $F_{1,13} = 0.02$, $p = 0.88$; abundance: $F_{1,13} = 0.08$, $p = 0.78$). Collector-gatherers were the most abundant FFG across all treatments, and comprised 49 – 66% of identified individuals (Fig. 4.2C, Appendix B), but biomass and abundance did not change with salamander biomass (biomass: $F_{1,13} = 2.29$, $p = 0.15$; abundance: $F_{1,13} = 0.77$, $p = 0.40$). Macroinvertebrate predators were also not affected by salamander biomass (biomass: $F_{1,13} = 0.26$, $p = 0.62$; abundance: $F_{1,13} = 0.54$, $p = 0.47$)

We did not detect any effects of macroinvertebrates on detrital resources. Collector-gatherer biomass was not related to standing stocks of FBOM ($F_{1,7} = 0.32$, $p = 0.59$), and shredder biomass was not related to standing stocks of CBOM ($F_{1,9} = 1.42$, $p = 0.27$), nor was *k* correlated with shredder biomass ($F_{1,9} = 0.46$, $p = 0.52$). Predatory macroinvertebrate biomass was positively correlated with non-predatory macroinvertebrate biomass ($F_{1,24} = 27.11$, $p < 0.001$), which indicates that consumption by predators was not reducing the biomass of other macroinvertebrates.

Despite generally dry conditions during the experiment, a large rain storm (161 mm in 24 hours) on day 62 of the experiment increased streamflow and overturned baskets in 6 enclosures, resulting in measurements of detrital standing stocks in only 9 enclosures on day 63. Across all enclosures, mean CBOM standing stocks were higher than standing stocks of FBOM (CBOM: 1019 ± 55 g AFDM m^{-2}; FBOM: 853 ± 185 g AFDM m^{-2}). However, neither CBOM nor FBOM differed as a function of salamander biomass (CBOM: $F_{1,7} = 0.12$, $p = 0.74$; FBOM: $F_{1,7} = 0.001$, $p = 0.98$).

Effects on function

Basket loss during the high flow event also reduced the number of replicates for measurements of ecosystem function (n = 9). Eight of 9 chambers exhibited net NH_4 uptake, with a mean uptake rate of 23.98 ± 7.00 µg N m^{-2} min^{-1}. Salamander biomass was negatively correlated with NH_4 uptake rate (Fig. 4.3, Table 4.2). Water temperature was also retained in the top model for NH_4 uptake, despite varying little across chambers (range: 12.9 – 13.2°C; Table 4.2). The intercept-only model for NH4 uptake also had substantial support (Table 4.2).

Measured respiration ranged from 0.12 – 1.47 g O_2 m^{-2} min^{-1}, and was not significantly related to salamander biomass nor organic matter standing stocks (Table 4.2). None of the predicted drivers were significantly related to chamber respiration, as only the intercept-only model had substantial support ($AIC_c \leq 2$; Table 4.2).

Leaf breakdown rate, *k*, differed among enclosures (range: 0.0091 – 0.0146), but was not affected by salamander abundance ($F_{4,10} = 0.10$, $p = 0.98$) or biomass (Fig. 4.4). Across experimental enclosures, leaf packs lost an average of 57.8 ± 1.4% of their mass by day 63.

Discussion

Despite relatively high salamander biomass in the 10- and 15-salamander treatment enclosures, we did not detect consumptive effects of salamanders on macroinvertebrates, nor cascading effects on organic matter standing stocks or rates of respiration or *k*. However, we did detect a correlation between salamander biomass and NH_4 uptake, which suggests that salamanders might affect ecosystem functions via localized nutrient regeneration. Using previously published relationships between Dq SVL and NH_4 excretion rates (Keitzer and Goforth 2013b), we estimated salamander NH_4 excretion by summing excretion estimates for individuals in each enclosure measured on day 63. Estimated salamander excretion per enclosure ranged from 0 to 2.50 μg N m^{-2} min^{-1}, which corresponds to 4.8 ± 1.1% of measured NH_4 uptake. While our enclosures focused on salamander effects during the transition from summer to fall (August – October), previous work has found that the proportion of nitrogen demand collectively fulfilled by Dq and Ew excretion is seasonally variable, and decreases after leaf-fall (Keitzer and Goforth 2013b). In early September, prior to leaf-fall, excretion by Dq and Ew larvae contributed 10% of reach-scale nitrogen demand, but contributed less than 4% in mid-November after leaf fall (Keitzer and Goforth 2013b), when uptake velocities increase due to increased respiration of freshly fallen, low-nitrogen leaf detritus (Valett et al. 2008, Gibson and O'Reilly 2012). Because we only manipulated Dq biomass, we were unable to account for the NH_4 demand fulfilled by Ew. Our estimate of salamander excretion on NH_4 uptake also does not consider total nitrogen demand by headwater streams, and excretion of urea and NH_4 by larval salamanders can contribute over 28% of stream nitrogen demand, while salamander bodies constitute large pools of stored nutrients, including nitrogen (Milanovich et al. 2015). Accordingly, reduced salamander biomass could alter stream nitrogen dynamics, both through

decreased cycling through excretion and by reducing nitrogen storage in salamander body tissues (Milanovich et al. 2015).

We did not detect consumptive effects of salamanders directly on their prey, and thus did not detect a trophic cascade on basal resource standing stocks or ecosystem rates two and three trophic levels removed. Salamanders did not significantly change macroinvertebrate community composition (Fig. 4.2A), and contrary to our expectation, macroinvertebrate shredder and salamander biomasses were not inversely correlated. Previous studies of Dq diets in Coweeta streams found that shredders comprised ~26% of prey biomass (Bumpers 2014), and that there was a significant relationship between salamander SVL and prey biomass in Dq guts ($mg\ prey\ AFDM = 0.119(SVL) - 1.748$, $R^2 = 0.18$; Bumpers 2014). Based on that relationship and measured salamander SVLs in our enclosures, salamanders could consume 2.67 to 12.16 mg shredder dry mass per day. While this estimate of prey biomass exceeds the observed shredder biomass in day-63 leaf packs (Table 1, Appendix B), salamanders would be expected to feed within the whole enclosure, rather than strictly within the leaf packs. However, the lack of observed effect of salamander biomass on shredders in the leaf packs, combined with the observation that our leaf breakdown rates (mean: 0.012 ± 0.000; range: 0.0091 – 0.0146) closely matched mean rates previously reported for *A. rubrum* across multiple headwater streams at Coweeta (0.011 ± 0.001; Manning 2015) suggests that macroinvertebrate immigration and emigration (Cooper et al. 1990) compensated for salamander predation inside enclosures.

Our inability to detect relationships between Dq biomass on macroinvertebrate biomass, abundance, or FFG composition is consistent with previous salamander work at Coweeta that only detected reductions in macroinvertebrate biomass when enclosures included mixed communities of salamanders (Keitzer and Goforth 2013a). In their study, Keitzer and Goforth

(2013a) found that the combined treatment of Dq and Ew reduced overall macroinvertebrate abundance, and in particular the abundance of Chironomidae, compared to monoculture treatments of either salamander species, which did not significantly differ from salamander-free controls. While Ew larvae were present in our study stream, we did not enclose Ew individuals due to their small size at the onset of our experiment (current study: August, versus November in Keitzer and Goforth 2013a). In addition, our enclosures contained seasonally relevant standing stocks of leaf litter (~100 g m^{-2}), which may have reduced our ability to detect salamander consumptive effects on macroinvertebrates in individual leaf packs, compared to the very low litter biomass stocked by Keitzer and Goforth (2013a; 12 g m^{-2}), which could have artificially concentrated macroinvertebrates and salamander predation within the enclosure. Future work that explicitly quantifies biomass-specific effects of salamander species on macroinvertebrate abundance across seasons will help resolve the role of Dq versus Ew in affecting macroinvertebrates.

The seasonal timing of experiments to quantify consumptive effects of salamanders on ecosystem structure and function likely also plays an important role in the detection and magnitude of those effects, as has been seen with other macroconsumers. While we are not aware of studies to date on the seasonal effects of salamander predation in streams, two vertebrate predators in Coweeta streams, mottled sculpin (*Cottus bairdii*) and brook trout (*Salvelinus fontinalis*) are known to vary seasonally in their effects on macroinvertebrate biomass and community structure (Cheever and Simon 2009). Cheever and Simon (2009) found that there were effects of season on macroinvertebrate community structure, as well as fish-by-season interactions on macroinvertebrates, with most macroinvertebrates having their highest densities in summer. As in our enclosure study, Cheever and Simon (2009) failed to detect a top-

down predator effect that propagated, in their case, to algal abundance, despite a seasonal reduction in grazing macroinvertebrates. However, some macroconsumers have consistent effects on ecosystem structure and function regardless of season. In both summer and autumn, crayfish accelerated leaf breakdown rates, though this effect was via direct shredding of leaf material rather than through an intermediate change in macroinvertebrate biomass (Schofield et al. 2001). Studies that explicitly test the magnitude of salamander, fish, and crayfish effects across seasons and species combinations will help resolve their importance in structuring macroinvertebrate communities.

Several logistical challenges could have contributed to our failure to detect salamander effects on macroinvertebrates or ecosystem rates. The large rainstorm the day before chamber measurements increased stream discharge, which toppled enclosure baskets and substantially reduced our sample size and statistical power to detect salamander effects. More generally, enclosure design can affect the detection of stream predator effects on prey populations, in that studies failing to detect consumer effects tend to have significantly greater enclosure mesh sizes than studies reporting significant consumer effects (Cooper et al. 1990). In the present study, our mesh size was ~1.5 mm, while the mesh sizes used in previous studies that detected salamander effects on macroinvertebrates were larger (2 mm: Keitzer & Goforth, 2013; 5 mm: Nery & Schmera, 2016). While we might have thus expected a greater likelihood of detecting a top-down effect, our small mesh size may have excluded larger macroinvertebrates from entering the enclosures. In addition, while the synthesis by Cooper et al. (1990) did not find a significant difference in enclosure duration between studies that did (23 ± 10 days) or did not (25 ± 11 days) detect predator effects, those mean experimental durations were less than half of the present study. Compared specifically to larval salamander studies, our enclosure period was over twice

as long (56 days) as experiments that have detected effects on macroinvertebrate abundance (25 days: Keitzer & Goforth, 2013; 10 days: Nery & Schmera, 2016). However, all of these short-term enclosure studies, including our own, may misrepresent long-term effects of salamander biomass changes, as long-term changes in the structure and function of tropical streams following the loss of tadpoles varied when quantified 2, 6, or 8 years after tadpole decline (Rantala et al., 2015 and references therein).

Despite an incomplete understanding of the roles of salamanders in structuring macroinvertebrate communities and affecting ecosystem functions, there is an increasing awareness of the threats to salamander populations and communities, particularly in the southeastern United States, due to climate and land use changes. The southeastern United States is predicted to experience changes in precipitation timing and intensity (Bates et al. 2008), and Dq larvae have decreased body condition during droughts (Currinder et al. 2014), while survival probability of both adult and larval Dq decrease with increasing drought duration (Price et al. 2012). Habitat models predict that the availability of suitable salamander habitat will decline as soon as 2020 due to climate change throughout the Appalachian Highlands region (Milanovich 2010). In addition, land use change and a lack of protection for headwater streams, where salamanders have high abundance and small home ranges (Peterman et al. 2008), can contribute to an overall reduction in habitat, and likely, salamander populations. This threat underscores the importance of continued work to understand the nuanced roles of salamanders in streams, in order to anticipate ecosystem-level changes if their populations decline.

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Table 4.1 Summary of macroinvertebrate metrics by functional feeding group (FFG) on day 63 grouped by initial salamander treatment (Trt.; individuals per enclosure). Salamander (Dq) biomass was estimated based on measured snout-vent-lengths (SVL) for salamanders recovered on day 63, and a length-mass regression for Dq in Coweeta streams (P. Bumpers, unpublished data). Macroinvertebrates were classified as collector-gatherers (CG), filter feeders (FF), predators (P), scrapers (Sc), or shredders (Sh) according to Merritt et al. (2008). Macroinvertebrate values are biomass (mg dry mass); values in parentheses are abundance (individuals). Total macroinvertebrates include all FFGs (including collected pupa, which were counted toward totals but not assigned a FFG). All values are mean ± 1 SE of three enclosures per treatment.

Trt	Dq biomass ($g\ m^{-2}$)	CG	FF	P	Sc	Sh	Total macroinvertebrates
0	0.00 ± 0.00	0.65 ± 0.42 (12.4 ± 5.5)	0.17 ± 0.06 (1.0 ± 0.0)	0.87 ± 0.33 (7.0 ± 2.5)	NA	3.55 ± 2.66 (6.0 ± 1.1)	4.48 ± 2.80 (25.2 ± 8.3)
3	3.87 ± 0.04	0.62 ± 0.19 (18.2 ± 5.2)	NA	0.92 ± 0.47 (4.3 ± 0.6)	NA	3.89 ± 1.48 (3.3 ± 1.0)	5.32 ± 1.78 (22.8 ± 5.8)
5	4.46 ± 0.33	0.60 ± 0.13 (16.3 ± 4.9)	0.07 ± 0.02 (1.0 ± 0.0)	0.75 ± 0.37 (6.3 ± 2.8)	1.32 ± 0.00 (1.0 ± 0.0)	1.64 ± 0.84 (2.5 ± 0.6)	3.24 ± 1.18 (26.0 ± 7.1)
10	12.25 ± 0.00	0.83 ± 0.30 (17.0 ± 8.2)	0.78 ± 0.52 (2.0 ± 1.0)	0.50 ± 0.24 (6.0 ± 1.2)	0.54 ± 0.15 (1.0 ± 0.0)	2.13 ± 1.15 (4.3 ± 1.7)	2.94 ± 1.20 (24.6 ± 10.9)
15	18.43 ± 0.93	1.05 ± 0.18 (26.8 ± 7.8)	0.98 ± 0.00 (5.0 ± 0.0)	0.80 ± 0.35 (8.8 ± 2.4)	0.49 ± 0.00 (1.0 ± 0.0)	4.00 ± 2.21 (10.0 ± 2.3)	6.10 ± 2.68 (46.7 ± 12.8)

Table 4.2. Linear models and parameter estimates for relationships between chamber rates (ecosystem respiration, ammonium uptake) and hypothesized drivers (salamander biomass [g m^{-2}], chamber water temperature [°C], coarse and fine benthic organic matter [CBOM, FBOM; g AFDM m^{-2}]). Model selection was based on AIC_C values. Hypothesized drivers and rates were natural log (x + 1) transformed to meet assumptions of normality. The number of parameters (*K*), AIC_C score, change in AIC_C from the top model (ΔAIC_C), weight and cumulative weight of support (W_i, $CumW_i$), log likelihood (LL), and R^2 are reported for models with substantial support ($\Delta AIC_C \leq 2$; only one model per rate), along with estimates of the intercept and slopes, standard errors (SE) and P-values. P-values in bold indicate statistical significance ($\alpha = 0.05$) while italicized P-values were marginally significant ($\alpha = 0.10$).

Model	*K*	AIC_C	ΔAIC_C	W_i	$CumW_i$	LL	R_c^2	Estimate	SE	P
Ecosystem respiration										
Intercept only	2	29.24	0	0.50	0.50	-11.62	-			
Intercept								-0.70	0.31	*0.056*
Ammonium uptake										
Biomass + Temperature	4	83.62	0	0.57	0.57	-32.81	0.71			
Intercept								7725.73	2068.55	**0.010**
ln (Salamander biomass + 1)								-24.81	5.40	**0.004**
ln (Temperature)								-2980.74	802.31	**0.010**
Intercept only	2	85.27	1.65	0.25	0.82	-39.64	-			
Intercept								23.98	7.00	**0.009**

Figure Legends

Fig. 4.1 Conceptual diagram of predicted salamander effects on ecosystem respiration and ammonium uptake. Solid lines and arrows indicate direct consumptive effects while dashed lines and arrows indicate indirect enrichment effects.

Fig. 4.2 Macroinvertebrate (A) community composition among salamander treatments, (B) functional feeding group (FFG) biomass (mg dry mass per gram leaf pack AFDM), and (C) FFG abundance (individuals per gram leaf pack AFDM). Lines in (A) indicate convex hulls of macroinvertebrate families for each salamander treatment (solid black: 0; solid gray: 3; dashed black: 5; dashed gray: 10; dotted black: 15 individuals per enclosure) based on non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity index. Letters in (B) and (C) indicate significant ($\alpha = 0.05$) differences between FFGs. Salamander treatments are grouped in (B) and (C) as there were no significant differences between FFG biomass or abundance as a function of salamander biomass. See Table 4.1 for FFG abbreviations.

Fig. 4.3 Rates of ammonium (NH_4) uptake as a function of larval *Desmognathus quadramaculatus* biomass in each enclosure on day 63. Each point represents a single enclosure ($n = 9$). Dashed horizontal lines indicates zero net uptake; negative values indicate net NH_4 mineralization, while positive values indicate net NH_4 uptake. Solid line indicates significant ($\alpha = 0.05$) fit from the top ($\Delta AIC_C \leq 2$) linear mixed effects model (see Table 4.2).

Fig. 4.4 Leaf breakdown rate (*k*) as a function of salamander biomass in enclosures on day 63. Dashed line and gray box indicate mean ± 1 SE of previously quantified *Acer rubrum* breakdown rates in Coweeta streams (Manning 2015).

Fig. 4.1

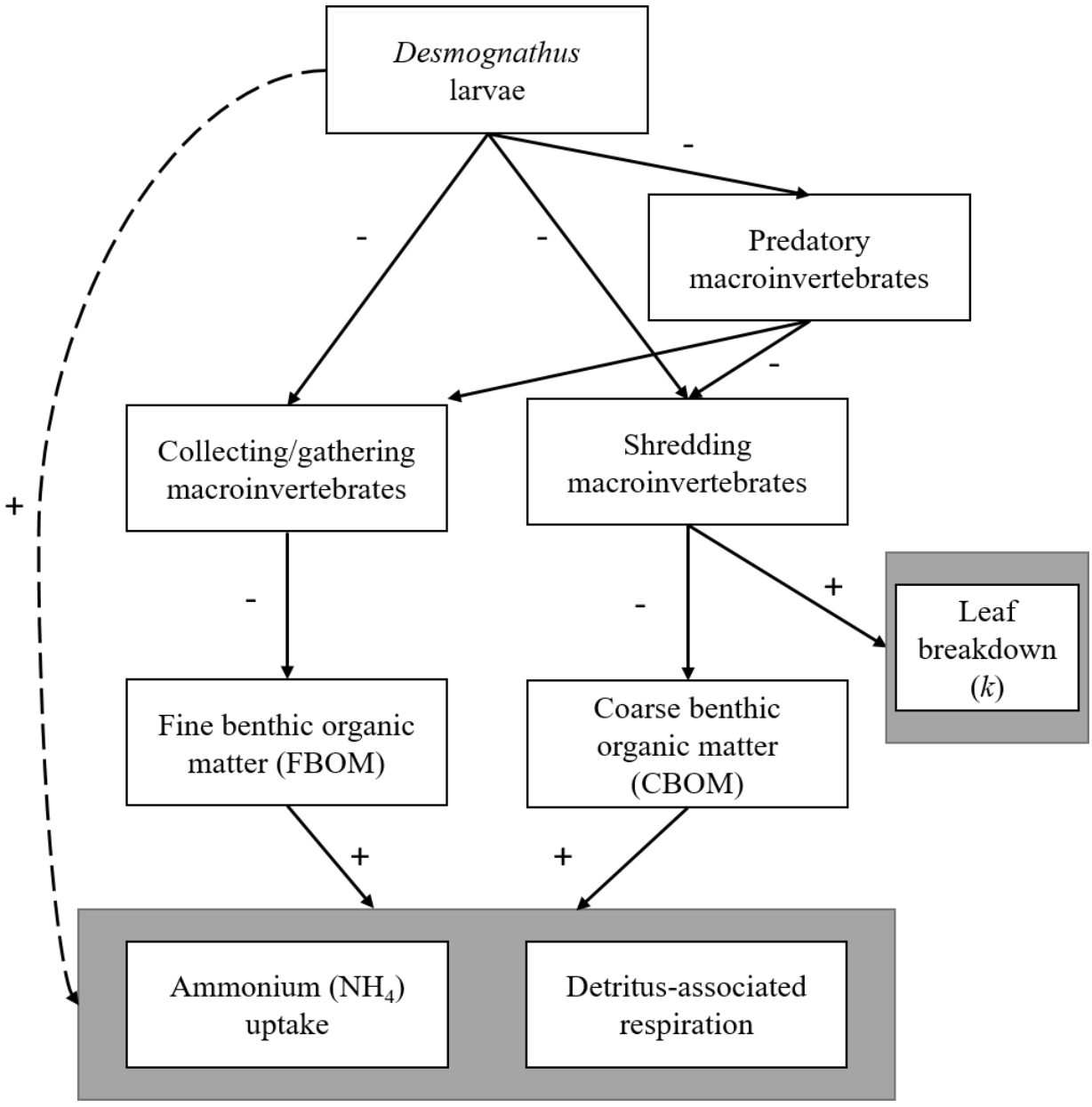

Desmognathus larvae
Predatory macroinvertebrates
Collecting/gathering macroinvertebrates
Shredding macroinvertebrates
Leaf breakdown (*k*)
Fine benthic organic matter (FBOM)
Coarse benthic organic matter (CBOM)
Ammonium (NH_4) uptake
Detritus-associated respiration
-
-
-
-
-
+
+
+
+
+

Fig. 4.2

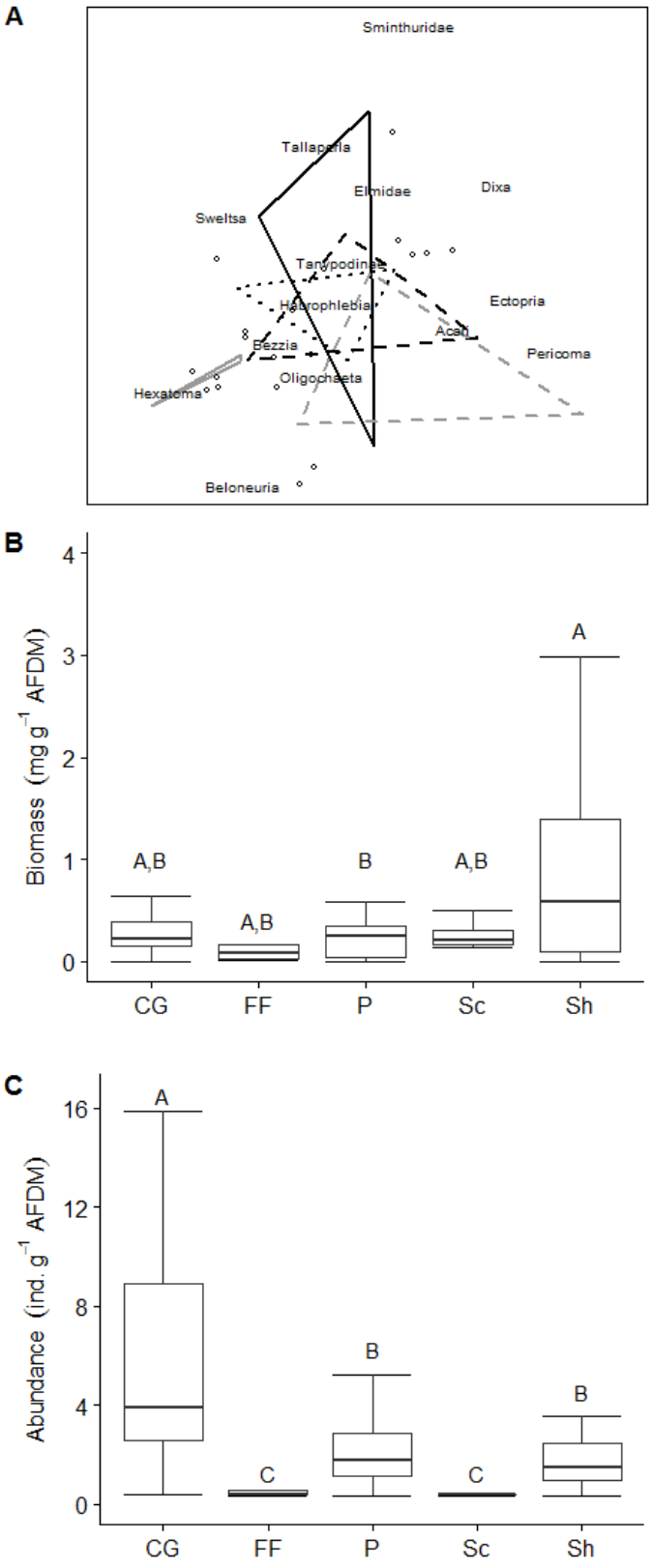

A
Sminthuridae
Tallaperla
Elmidae
Dixa
Sweltsa
Tanypodinae
Ectopria
Hydrophlebia
Acari
Bezzia
Pericoma
Oligochaeta
Hexatoma
Beloneuria
B
Biomass (mg g^{-1} AFDM)
0
1
2
3
4
A,B
A,B
B
A,B
A
CG
FF
P
Sc
Sh
C
Abundance (ind. g^{-1} AFDM)
0
4
8
12
16
A
C
B
C
B
CG
FF
P
Sc
Sh

Fig. 4.3

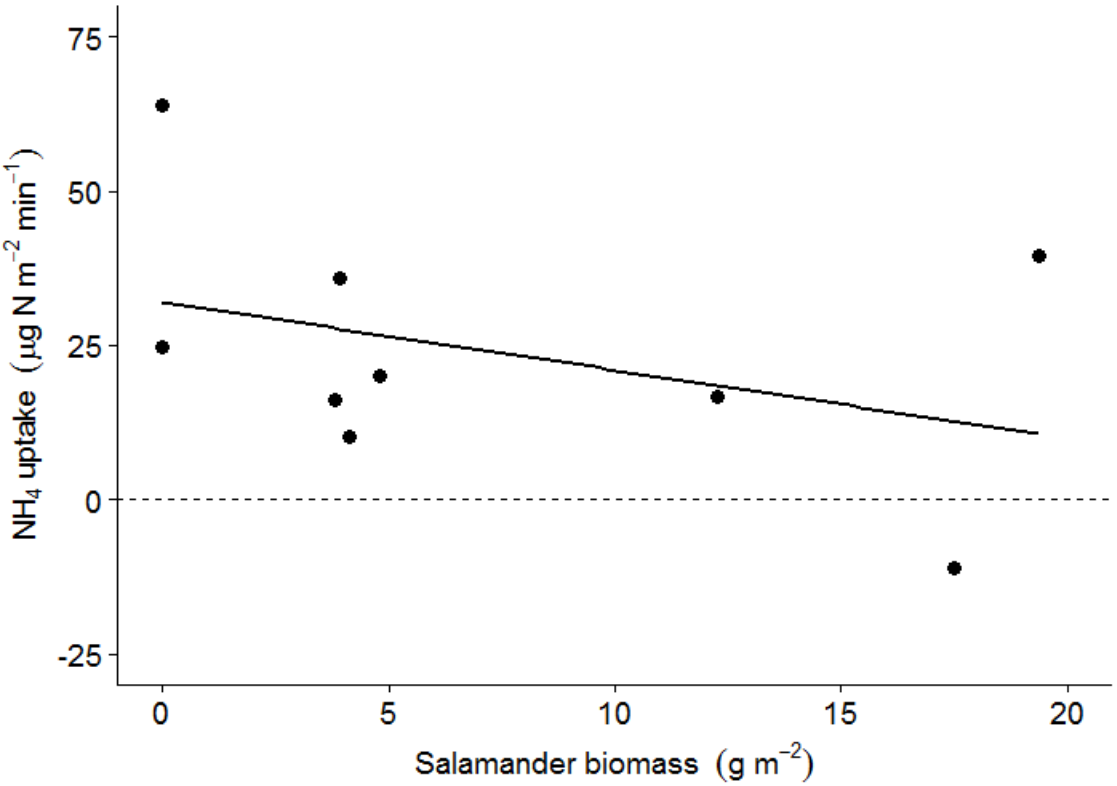
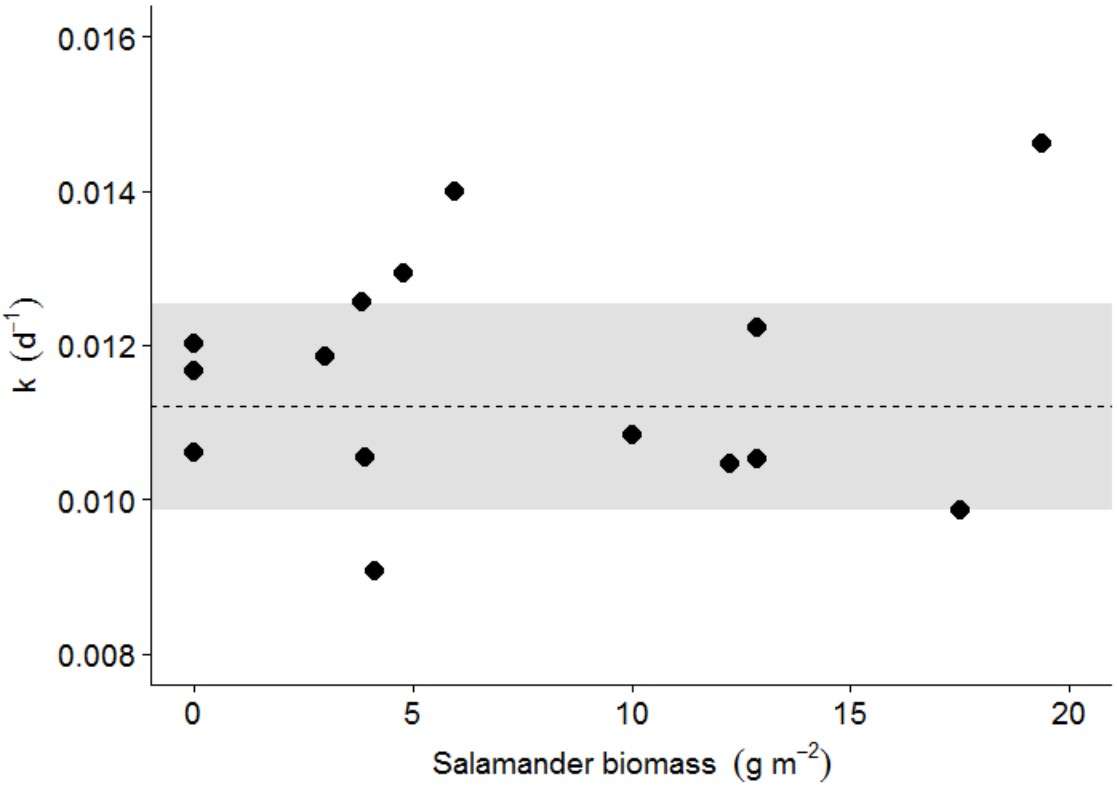

75
50
25
0
-25
0
5
10
15
20
NH4 uptake (µg N m-2 min-1)
Salamander biomass (g m-2)

Fig. 4.4


0.016
0.014
0.012
0.010
0.008
k (d^{-1})
0
5
10
15
20
Salamander biomass (g m^{-2})

CHAPTER 5

COMPARING EFFECTS OF AQUATIC MACROCONSUMERS IN DIVERSE HEADWATER STREAM NETWORKS[4]

[4] Farrell, K.J., A.D. Rosemond, J. Rüegg, C.L. Baker, L.E. Koenig, S.P. Parker, C. Song, M.T. Trentman, K. Gido, M.B. Flinn, M.R. Whiles, and W.H. McDowell. To be submitted to *Ecosystems*.

Abstract

Trophic downgrading is leading to shortened food chain lengths and simplified food webs in ecosystems worldwide. In freshwater ecosystems, top consumers (macroconsumers) are at risk of extirpation due to land use change, nutrient pollution, overharvesting, and climate change. To assess the net effects (i.e., of consumption, nutrient excretion) of reduced macroconsumer community biomass on ecosystem functions, we used paired consumer removal and control patches across five river networks from distinct biomes in areas relatively unimpacted by human activities. We tested whether ecosystem rates (ecosystem respiration [ER], gross primary production [GPP] and ammonium [NH_4] uptake) or structures (coarse [CBOM] and fine [FBOM] benthic organic matter, chlorophyll *a* [chl-a], and macroinvertebrate biomass) differed between exclusion and control patches. Across the five networks, we found that ER was driven by both CBOM and FBOM, while chl-a was positively correlated with GPP, but that rates did not significantly differ between control and exclusion patches. Macroinvertebrate and macroconsumer biomasses were generally not related to the standing stocks of their food resources. However, we did detect an inverse relationship between omnivorous macroconsumers and benthic organic matter standing stocks, though our design was unable to assess whether consumption and/or bioturbation drove that pattern. Predatory macroinvertebrate biomass was positively correlated with total macroinvertebrate biomass, which provides additional support for bottom-up control in these systems. Within the limits of our short-term exclusion, our findings suggest that across diverse stream networks, resource availability controls both ecosystem processes and consumer standing stocks, and that these streams are more strongly bottom-up (resource) than top-down (consumption) controlled.

Introduction

Human-induced changes in freshwater ecosystems, through altered flow and thermal regimes, habitat degradation, and nutrient loading, can change the species composition of stream communities (Dudgeon et al. 2006, Woodward et al. 2010). Large aquatic consumers (hereafter, macroconsumers), including fishes, crustaceans, and amphibians, are considered particularly vulnerable (Duffy 2003, He et al. 2017), with extinction rates estimated to be over five times higher than for species in terrestrial systems (Ricciardi and Rasmussen 1999), while the frequency of community alterations through macroconsumer species losses is expected to increase with ongoing climate change (Perkins et al. 2010, Woodward et al. 2010).

Aquatic macroconsumers are known to play a number of unique roles in streams. Macroconsumers can shape prey communities via consumption and the initiation of trophic cascades (e.g., Power 1990, Dahl 1998, Atwood et al. 2013), affect nutrient availability and ecosystem productivity via excretion of limiting nutrients (e.g., Vanni 2002, Small et al. 2011, Capps and Flecker 2013, Atkinson et al. 2016), and accelerate organic matter and nutrient transport via bioturbation (e.g., Vanni 2002, Taylor et al. 2006, Gido and Bertrand 2010). While each of these roles can be assessed independently for individual organisms, experiments that consider the net effects of macroconsumer communities through the lens of carbon and nutrient processing can provide new insights into the ecosystem-level roles of macroconsumers (Schmitz et al. 2014).

Previous research to identify when and how macroconsumers have significant detectable effects on stream ecosystems has produced mixed results as to the importance of consumers in altering ecosystem structure and function. The ability to detect macroconsumer effects appears to be dependent in part on consumer biomass and feeding mode. Algivorous and detritivorous

macroconsumers can directly reduce algal and organic matter standing stocks, respectively, through consumption (e.g., Power et al. 1985), but also stimulate algal productivity or detrital breakdown via nutrient excretion (Taylor et al. 2006, Small et al. 2011, Capps et al. 2014), and examining both pathways simultaneously can provide insight into consumer net effects (Capps et al. 2014). Insectivorous and predatory macroconsumers can also have consumptive effects, which can trigger trophic cascades on basal resource standing stocks and ecosystem processes by reducing primary consumer abundance (Flecker 1984, Power et al. 1985, Power 1990, Dahl 1998, Rosemond et al. 2001, Ruetz et al. 2002, Gibson et al. 2004) or altering the behavior of primary consumers (Flecker and Townsend 1994, McIntosh and Townsend 1996, Schmitz et al. 2008). However, reductions in macroinvertebrate biomass by insectivorous macroconsumers do not always result in detectable trophic cascades via changes in algal biomass or organic matter standing stocks (Ruetz et al. 2004, Cheever and Simon 2009), and may not result in detectable changes in macroconsumer prey itself (Allan 1982, Reice 1991, Ho and Dudgeon 2016). The lack of consistent detectable macroconsumer effects in these studies emphasizes that the net effects of macroconsumers in streams may be masked by high turnover rates of basal resources and prey (Cooper et al. 1990).

To date, many assessments of stream macroconsumer roles have focused on experimentally changing the abundance or density of a single species in a single stream reach (but see Rosemond et al. 2001, Cheever and Simon 2009, Ho and Dudgeon 2016), and tests of the generality of macroconsumer effects within stream networks and across systems are limited (but see Dahl and Greenberg 1996, Atwood et al. 2013). In addition, many studies that have identified significant macroconsumer effects on ecosystem processes have been from systems with relatively high macroconsumer abundance and biomass (e.g., Power et al. 1985, Taylor et

al. 2006, McIntyre et al. 2008, Capps and Flecker 2013), though studies with high macroconsumer densities have also failed to detect top-down effects (Ho and Dudgeon 2016). Finally, few studies have assessed macroconsumer effects on both autochthonous and allochthonous (detritus-based) pathways (but see Woodward et al. 2008), despite contributions of both pathways to energy budgets of most streams.

To test some of these ideas, we reduced the density and biomass of mixed macroconsumer communities in three reaches within stream networks from five distinct biomes as part of the "Scale, Consumers, and Lotic Ecosystem Rates" (SCALER) project, to assess top-down effects on measures of ecosystem structure and function. As sampled biomes ranged from tropical rainforest to Arctic tundra, the identity, abundance, and trophic groups of macroconsumers varied both within and among stream networks, from a community composed of a single insectivorous fish species in the Arctic streams (Deegan et al. 1997), to a more diverse community of omnivores, algivores, and insectivores in prairie (Dodds et al. 2004) and forested streams (Freeman et al. 1988, Pringle et al. 1999). Within some networks, barriers to migration (e.g., waterfalls) resulted in fishless macroconsumer communities in headwater streams, where crustaceans (shrimps, crayfishes) and/or amphibians (larval salamanders) were the dominant macroconsumers. Despite differences in the biotic communities and abiotic templates of our study streams, the consistent experimental design across sites allowed us to compare the effects of consumer trophic group and density on a common suite of basal resource standing stocks and ecosystem rates.

Here, we present data on the top-down consumptive effects of macroconsumers on ecosystem rates (GPP, ER, NH_4 uptake) via changes in macroinvertebrate biomass and community structure, and basal resource standing stocks. Across sites, we predicted that

consumer effects would depend on trophic position and functional feeding group, and that macroconsumer biomass would be positively correlated with any observed consumer effects (Fig. 5.1). Specifically, we predicted that if insectivorous macroconsumers reduce the biomass of macroinvertebrates that feed on standing stocks of coarse (CBOM) and fine (FBOM) benthic organic matter, then insectivore biomass would be positively correlated with ecosystem respiration (ER). Similarly, we expected that if insectivorous macroconsumers reduce the biomass of grazing macroinvertebrates, and that grazing invertebrates exert top-down control on algal biomass, then insectivore biomass would be positively correlated with gross primary production (GPP) via increased algal standing stocks. In contrast, we hypothesized that if algivorous macroconsumers feed on biofilms, then their biomass would be negatively correlated with GPP (Fig. 5.1). We tested these predictions using paired consumer exclusion and control cages in three streams within each biome by first assessing the relationship between measured ecosystem rates (ER, GPP, NH_4 uptake) and predicted rate drivers (CBOM, FBOM, chlorophyll-a), and then determining whether macroconsumers affected driver standing stocks via trophic cascades.

Methods

Study sites

Our study included five headwater stream networks across the United States that encompassed distinct biomes: tropical forest, deciduous forest, tallgrass prairie, boreal forest, and tundra (Fig. 5.2; see Rüegg et al. 2015b for full site descriptions). Experiments were conducted sequentially across sites between January and August 2013 (Table 5.1).

Consumer exclusion at the patch scale

Within each biome, we established *in situ* paired consumer exclusion and control mesocosms (hereafter: patches) in three streams along a discharge gradient (Fig. 5.2). Patches were constructed as square wooden frames divided into two triangular sections (.12 m^2 each). All sides of one triangle were enclosed by 6-mm wire mesh hardware cloth (exclusion), while the other triangle had one side open to consumer access (control). Hardware cloth also covered the bottom of all patches to prevent burrowing macroconsumers from entering the patches from the bottom. Patches (n = 8 per stream) were installed by removing substrates from the stream bottom, installing the patches flush with the benthos, and anchoring in place with rebar along the upstream vertex. Inside each side of a patch, we installed sets of five plastic mesh baskets (0.01 m^2 each) filled with representative, colonized stream substrates (mixed gravels and cobbles) collected from within the stream. Additional substrates were placed around the baskets so that baskets were flush with the surrounding stream bottom. Patches were maintained for ~40 days each, with daily or twice-daily cleaning of the mesh as needed to remove accumulated organic matter and maintain water flow.

Rate measurements

Recirculating chambers were used to quantify patch-scale metabolism and NH_4 uptake at the end of the experimental period (see Rüegg et al. 2015 for detailed methods). Briefly, three baskets from one side of a patch were run in a chamber, with six chambers run simultaneously on the streambank adjacent to each study reach. Each chamber was fitted with a YSI ProODO sensor (Yellow Springs, Ohio, USA) and an Odyssey light recorder (Dataflow Systems Limited, Christchurch, New Zealand), which both logged at 1-minute intervals. Ecosystem respiration

(ER) measurements were quantified in covered chambers for 15 minutes, followed by an additional 15 minutes of net ecosystem production (NEP) in ambient light conditions with the chamber cover removed.

Following NEP measurements, DO and light loggers were removed and a 3 mL slug of NH_4Cl stock solution was added to each chamber to achieve a target chamber-water ammonium concentration of 3-5× background concentrations, or 25 μg L^{-1} NH_4-N, whichever was higher based on ambient NH_4 concentrations. Water samples were withdrawn from the chambers 1, 4, 10, 20, and 40 minutes post-slug addition to quantify ammonium uptake in each chamber. Collected water samples were immediately filtered through a 0.7 μm glass fiber filter (GF/F, Sterlitech Corporation, Kent, Washington, USA), cooled on ice, and frozen upon return to the laboratory. After the 40-minute sample was collected, baskets and substrates were removed from chambers, and chambers were thoroughly rinsed in stream water before initiating another run. In the lab, frozen samples were defrosted and immediately analyzed for NH_4-N using the colorimetric phenol-hypochlorite method (APHA 1995) and read on a spectrophotometer (Shimadzu UV-1800) at 640 μm wavelength.

Organic matter quantification

Organic matter standing stocks were quantified from the two patch baskets in each set that were not run in chambers. One basket was used to collect chl-a and FBOM; the basket was removed from the stream and quickly placed into a bucket with 5 L of stream water. Surface substrates were removed and placed in a polyethylene bag for chl-a analysis. Remaining contents of the basket were then dumped into the water, agitated, and a 500-mL subsample was collected to quantify FBOM. Coarse benthic organic matter and macroinvertebrates were quantified from

the remaining basket. The basket was removed from the stream by placing it onto a 250 μm sieve and transferring the basket to an enamel pan. Substrates were rinsed with streamwater and removed, and remaining materials (CBOM and macroinvertebrates) were rinsed into polyethylene bags and preserved with 8% formalin.

In the laboratory, FBOM slurry was filtered through a pre-weighed 0.7 μm GF/F until it clogged. Filtered volume was recorded and filters were dried at 50°C for at least 72 hours, and weighed for dry mass. Samples were then ashed at 500°C for 4.5 hours and reweighed for ash-free dry mass (AFDM). Chlorophyll-a was extracted from collected rocks using hot ethanol (Sartory and Grobbelaar 1984) and read using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) with an acidification correction for phaeophytin (APHA 2005, Parker et al. 2016). Chlorophyll-a was extrapolated to a per-area basis based on the surface area of scrubbed substrates, as estimated from either volume displacement-surface area relationships (LUQ, CWT, KNZ, ARC) or digital images of rock surfaces processed with ImageJ (CPC; Schneider et al. 2012). All macroinvertebrate and CBOM samples were processed by the Whiles lab at Southern Illinois University. Coarse benthic material was manually separated from macroinvertebrate samples under a dissecting microscope, then dried, weighed, and ashed as described for FBOM. Macroinvertebrates were identified to the lowest taxonomic level possible (typically genus) and measured to the nearest millimeter to estimate biomass based on previously published length-mass regressions (Benke et al. 1999). Functional feeding groups (FFGs) were assigned to each taxon based on groupings by Merritt et al. (2008).

Ecosystem rate estimation

Chamber rates of ER and GPP were estimated using a modification of methods described in Song et al. (2016), omitting the term for reaeration since gas exchange with the atmosphere through the chamber was minimal (Rüegg et al. 2015a). Briefly, we modeled the change in DO concentration over the logging interval as a mass balance between oxygen production (GPP) and consumption (ER), while incorporating a correction for light saturation (GPP; Jassby and Platt 1976) and an Arrhenius correction for temperature (ER, GPP; Parkhill and Gulliver 1999). The resulting equation for the change in DO concentration over time is:

$$\frac{d[O_2]}{dt} = P_{max} \tanh\left(\frac{\alpha I}{P_{max}}\right) e^{-\frac{E_{ap}}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)} - R_{T_0} e^{-\frac{E_{ar}}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)}$$

Where P_{max} is the light-saturated rate of photosynthesis, I is light intensity (PAR; μE m^{-2} s^{-1}), and α is the slope of the photosynthesis-light relationship at low light intensity, R_{T0} is the respiration rate at reference temperature T_0, T is the measured stream water temperature, and E_{ap} and E_{ar} are the activation energies of GPP and ER, respectively. Parameters for the model (P_{max}, α, $E_{ap,}$ R_{T0}, and E_{ar}) were estimated using a Bayesian approach; in forming the likelihood function, we assumed that the observation error between measured and modeled [O_2] was normally distributed. We interpolated light and temperature linearly between 1-minute logging intervals and solved for the estimated change in [O_2] using the "lsoda" function in the deSolve package for R (Soetaert et al. 2010). The prior for all parameters was a uniform distribution, and we used an adaptive random walk Metropolis-Hasting algorithm (Haario et al. 2001) to sample the posterior distribution. Estimates for each parameter were based on the mean of the posterior distribution. Estimated rates of ER and GPP were made by integrating modeled instantaneous rate estimates over the course of the entire chamber run (ER) or the ambient light period (GPP), and calculated

on an areal basis using the surface area of the chamber baskets (0.03 m^2) and the measured chamber water volume. All analyses were conducted using R 3.2.5 (R Core Team 2017).

Ammonium uptake was calculated as the linear change in ammonium concentration in chamber water over time:

$$Uptake = \frac{\Delta NH_4}{t} \times V \times A^{-1}$$

Where ΔNH_4 is the change in chamber ammonium concentration (μg L^{-1}), t is the elapsed time (min), V is the chamber volume (L), and A is the area of the chamber substrates (0.03 m^2). Estimates of NH_4 uptake were normalized to a background NH_4 concentration of 10 μg L^{-1}.

Consumer biomass estimation

At each stream within a biome, we estimated consumer biomass by blocking off reaches (50 – 150 m stream length) with 6-mm gridded mesh hardware cloth to prevent macroconsumer immigration or emigration. We captured aquatic macroconsumers (fishes, larval salamanders, crustaceans) within each reach using multiple-pass depletion, based on standard methods that were most appropriate for each biome and reach to maximize capture of individual macroconsumers (Appendix C). When depletion electroshocking was employed, a minimum of three passes with diminishing catch per pass were conducted to allow for population estimates of each captured species. During each pass, captured individuals were held in a water-filled bucket, then identified to species and measured (nose/rostrum to fork/fin or carapace length). To estimate consumer biomass from measured lengths, we used published length-weight relationships for individual species. Individuals were released back into the reach after consumer quantification was complete.

Estimates of total biomass per species in each reach were estimated for most biomes based on closed population removal method (Hayes et al. 2007 and references therin). In reaches where there were three removal passes with subsequent biomass depletion, we used maximum-likelihood estimators to predict capture efficiency (q) and population size (N). Biomass for reaches in KNZ could be reliably estimated using this method. In reaches where there was not a strong decline in biomass caught with increasing passes (LUQ, CWT), we adjusted our biomass estimation method by applying a q based on reaches within the biome where depletion was successful. In CPC and ARC, multi-pass depletion shocking was prohibited. In those biomes, visual observations of fish suggested that catch rates were high, so we applied a q of 0.8 and 1.0 for CPC and ARC, respectively.

Statistical analysis

To determine whether we could detect top-down effects of macroconsumers on ecosystem rates (ER, GPP, NH_4 uptake), we first built models to identify whether quantified basal resource drivers were significant predictors of rates (Fig. 5.1). Specifically, we tested whether CBOM and FBOM were significant predictors of ER, and whether chl-a was a significant predictor of GPP. We tested whether all three basal resource pools were related to NH_4 uptake. For each rate, we developed a suite of linear mixed effects models ('lme4' package for R; Bates et al. 2015) that included a nested random effect of patch within reach to account for the hierarchical nature of our experimental design. For ER, we used CBOM and FBOM as continuous fixed effects, and fixed effects of treatment (exclusion or control) and reach nested within biome. For each rate, the most complex model included additive effects of all fixed effects, and factors were removed iteratively, with the simplest model retaining only the

intercept. We used the 'AICcmodavg' package (Mazerolle 2016) to rank top models using AIC_c, and retained models with substantial support ($\Delta AIC_c \leq 2$, Burnham and Anderson 2004).

We tested for overall differences between macroconsumer exclusion and control patches on ecosystem rates (ER, GPP, NH_4 uptake) and basal resource standing stocks (CBOM, FBOM, chl-a) between biomes using a linear mixed effects model with an interaction between fixed effects of treatment and biome and a random effect of patch nested within reach. Significant effects were followed by post hoc comparisons between treatments using least-square means.

We then assessed whether macroinvertebrates and macroconsumers were affecting standing stocks of basal resources they are expected to feed on (Fig. 5.1) using linear mixed effects models. For macroinvertebrates, we tested whether CBOM was affected by shredders, whether FBOM was affected by collector-gatherers, and whether chl-a was affected by scrapers. For macroconsumers, we tested whether areal biomass of invertivores (omnivores + insectivores, and insectivores alone) was significantly related to total macroinvertebrates, or to individual FFGs that would initiate a trophic cascade to ER or GPP (Fig. 5.1). We also tested whether algivorous macroconsumers significantly affected chl-a. All macroinvertebrate and macroconsumer data were expressed as mass m^{-2}.

Finally, we tested whether macroinvertebrate community composition (as biomass per taxon) differed between exclusion and control patches within sites, among sites within biomes, and between biomes. We compared macroinvertebrate community biomass with non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity index in the 'vegan' package (Oksanen et al. 2017). In each stream, we summed macroinvertebrate biomass by taxonomic group (family for Insecta, otherwise order) across patches within a treatment, and built our community biomass matrix based on the summed values. We tested for significant

differences between macroinvertebrate communities in treatment and control patches using interactions between biome, reach within biome, and treatments using a permutational MANOVA based on the distance matrices ('vegan' function 'adonis'). All macroinvertebrate biomass values (mg DM m^{-2}) were natural log + 1 transformed prior to analysis.

Results

Macroconsumer biomass and trophic composition

Across all biomes and study reaches, macroconsumer areal biomass was between 0.07 and 6.49 g m^2 (Table 5.1). Total biomass was highest at the most downstream reach in KNZ and lowest at ARC, where only one of three experimental reaches had macroconsumers. Macroconsumers in most biomes were a mixture of omnivores (crustaceans, fishes) and insectivores (fishes, larval salamanders); algivorous macroconsumers were only present at LUQ and KNZ. At LUQ, one algivorous macroconsumer species was present (*Sicydium plumieri*, Erdman 1961), but was not a substantial contribution to overall biomass (~1% in both reaches where present), while in KNZ, algivorous macroconsumers, the fishes *Campostoma anomalum* and *Phoxinus erythrogaster* (Bertrand and Gido 2007), comprised over 50% of macroconsumer biomass in both sampled reaches.

Drivers of ecosystem rates

There were significant cross-biome patterns between basal resource standing stocks and measured ecosystem rates for both ER and GPP. Chamber ER was positively correlated with both CBOM (Fig. 5.3A) and FBOM (Fig. 5.3B) standing stocks, and differed among biomes (Table 5.2), with significantly lower ER at LUQ and CWT than at other sites (Table 5.3).

Similarly, GPP was positively correlated with chl-a in both top models (Table 5.2; Fig. 5.3C), and was different among biomes, with significantly lower GPP at LUQ and CWT than at other sites (Table 5.3). Ammonium uptake was not significantly related to any of the tested basal resource drivers; the only model with substantial support was an intercept-only model (Table 5.2). For all three measured rates (ER, GPP, NH_4 uptake), consumer treatment (exclusion vs. control) was not a significant predictor in any models with substantial support ($\Delta AIC_c \leq 2$; Table 5.2), and rates were not significantly different between macroconsumer exclusion and control patches (Fig. 5.4; ER: $F_{1,92} = 0.69$, $p = 0.41$; GPP: $F_{1,91} = 2.94$, $p = 0.09$; NH_4: $F_{1,71} = 2.00$, $p = 0.16$).

Effects of macroconsumer exclusion and macroinvertebrates on standing stocks

Standing stocks of CBOM were not significantly different between consumer treatments ($F_{1,104} = 0.10$, $p = 0.76$), but differed among biomes ($F_{4,10} = 12.91$, $p = 0.001$), with significantly higher standing stocks at CPC than at other sites (Table 5.3). There were significant effects of both consumer treatment and biome on FBOM, with higher standing stocks in exclusion than control patches ($F_{1,92} = 4.34$, $p = 0.04$), and higher standing stocks at KNZ and CPC than at other sites (Table 5.3; $F_{4,9} = 21.90$, $p < 0.001$). Chlorophyll-a did not differ between treatments ($F_{1,91} = 1.00$, $p = 0.32$), but was different among biomes, with significantly higher concentrations at KNZ (Table 5.3).

When we tested whether macroinvertebrate biomass was significantly related to presumed food resources across biomes (Fig. 5.1), we found that the biomass of shredding macroinvertebrates was not related to CBOM (Fig. 5.5A; $F_{1,56} = 1.91$, $p = 0.17$), but that shredder biomass was significantly lower at LUQ, where shredders were generally absent, than in other

biomes ($F_{4,10} = 30.56$, $p < 0.001$). Collector-gatherer biomass was not related to FBOM (Fig. 5.5B; $F_{1,101} = 0.14$, $F = 0.71$), and did not significantly differ among biomes ($F_{4,10} = 2.33$, $p = 0.12$). Chlorophyll-a was not related to macroinvertebrate scraper biomass (Fig. 5.5C; $F_{1,45} = 059$, $p = 0.45$), but scraper biomass was significantly higher at ARC and CWT than at KNZ ($F_{4,10} = 5.75$, $p = 0.01$). Macroinvertebrate predator biomass was positively correlated with total macroinvertebrate biomass (Fig. 5.5D; $F_{1,90} = 23.93$, $p < 0.001$), though neither predator biomass ($F_{4,10} = 0.81$, $p = 0.55$) nor total macroinvertebrate biomass ($F_{4,10} = 1.25$, $p = 0.35$) differed among biomes.

Detection of relationships between macroconsumers and their presumed food resources depended on macroconsumer trophic group. Omnivore biomass was significantly different among biomes (Fig. 5.6A; $F_{4,9} = 63.36$, $p < 0.001$), with the highest omnivore biomass at LUQ, and no omnivores present at CPC or ARC. Omnivore biomass was significantly negatively correlated with both CBOM standing stock (Fig. 5.6A; $F_{1,8} = 12.49$, $p = 0.008$) and FBOM standing stock (Fig. 5.6B; $F_{1,8} = 15.00$, $p = 0.005$), though our experimental design was unable to test whether consumption and/or bioturbation drove those patterns. Chlorophyll-a was not related to algivore biomass (Fig. 5.6C; $F_{1,1} = 12.09$, $p = 0.18$) when testing among biomes that had algivorous macroconsumers (LUQ, KNZ). Invertivore (omnivore + insectivore) biomass was significantly higher at LUQ than at CPC and ARC ($F_{4,9} = 9.77$, $p = 0.003$), but biomass of invertivores was not correlated with total macroinvertebrate biomass (Fig. 5.6D; $F_{1,8} = 0.03$, $p = 0.86$). Macroinvertebrate community composition was significantly different among biomes ($F_{5,10} = 25.23$, $p = 0.001$, $R^2 = 0.67$), but did not significantly differ between macroconsumer exclusion and control patches across sites within biomes ($F_{2,10} = 0.84$, $p = 0.67$, $R^2 < 0.01$).

Discussion

Across the SCALER networks, we found stronger evidence for bottom-up resource control of stream functions than macroconsumer control via consumption or nutrient enrichment, as benthic organic matter standing stocks drove measured ER and GPP, but rates were not significantly different between consumer exclusions and control patches. Benthic organic matter (CBOM, FBOM) was positively correlated with ER, and the observed range in CBOM and FBOM standing stocks across biomes was associated with a 40× increase in ER rates, from 0.14 – 5.67 mg O_2 m^{-2} min^{-1}. Similarly, GPP increased with chl-a concentration, and the observed range of chl-a across biomes was associated with an 87× increase in GPP, from 0.08 – 7.98 mg O_2 m^{-2} min^{-1}.

In contrast, we did not detect top-down macroconsumer consumption effects on ecosystem rates. While neither macroinvertebrate shredder nor collector biomass was correlated with standing stocks of their primary food source (CBOM, FBOM, respectively), omnivore biomass was negatively correlated with both CBOM and FBOM across sites. However, because there were no significant differences in ER between exclusion and control patches throughout the networks sampled (Fig. 5.4A), we conclude that the apparent omnivore effect may have been due to bioturbation, and that there was no detectable top-down control of ER in our reaches. Omnivores may 'dampen' the strength of top-down consumer effects (Strong 1992, Pringle and Hamazaki 1998, Rubbo et al. 2012), due in part to feeding on multiple trophic levels and reducing interaction strengths within the food web, though the presence of omnivores does not always reduce top-down control (reviewed in Pace et al. 1999, Shurin et al. 2006). Since chl-a did not differ between exclusion and control patches (Fig. 5.4B) and we failed to detect a relationship between chl-a and biomass of either grazing macroinvertebrates (Fig. 5.5E) or

algivorous macroconsumers (Fig. 5.5F), it appears that consumers do not exert significant top-down consumptive effects on primary production in our systems. Indeed, the detection of net top-down effects of macroconsumers may depend on macroconsumer biomass. Macroconsumer densities at our experimental sites were low compared to many previous studies that have detected strong net effects of consumer removal. The highest macroconsumer community biomass across our experimental sites was 6.49 g m^{-2}. In contrast, strong net effects by detritivorous *Prochilodus mariae* via consumption and bioturbation were identified when single species biomass was often in excess of 100 g m^{-2} (Taylor et al. 2006). Similarly, non-native algivorous Loricariid catfish had a net negative effect on algal biomass and ecosystem productivity, where measured biomass exceeded 200 g m^{-2} (Capps et al. 2014). However, high biomass is neither a guarantee nor a prerequisite for the detection of net macroconsumer effects; relatively high biomasses of algivorous (20.9 g m^{-2}) and predatory (29.7 g m^{-2}) fishes failed to result in net changes in ecosystem structure in Hong Kong Streams (Ho and Dudgeon 2016), while top-down effects via nutrient cycling have been detected by a characid fish with relatively low areal biomass (5.5 g m^{-2}; Small et al. 2011).

Isolating and detecting top-down consumer effects in lotic systems is challenging, whether using field manipulations, deletion experiments, or modeling, due in part to the open nature of stream ecosystems (Peckarsky et al. 2008). A primary limitation of our current approach was likely the relatively short duration (~30 days) of our consumer exclusions relative to the time over which we might have expected to detect macroconsumer effects on basal resource standing stocks (e.g., chl-a; Gido et al. 2010). However, increasing the duration of exclusions does not guarantee the detection of macroconsumer effects (e.g., Allan 1982), and longer-term exclusions can lead to nuanced conclusions about macroconsumer effects, as has

been seen when tracking changes in structure and function of tropical streams following the disease-induced extirpation of tadpoles (Whiles et al. 2013, Rantala et al. 2015). In addition to duration of exclusion, the use of mesh-sided enclosures and exclosures can affect the likelihood of detecting consumer effects, as detection of significant effects has been negatively correlated with mesh size (Cooper et al. 1990), and the mesh size used in SCALER streams (6 mm) is typical of experiments in which predator effects were not significant (Cooper et al. 1990). This may be due in part to mesh excluding large CBOM particles, including large leaf particles, which are a primary food source for many stream shredders (Wallace et al. 1982). The addition of experimental leaf packs (e.g., Ruetz et al. 2002), or an initial stocking of patches with ambient organic matter, could reduce that limitation, and might improve our ability to detect top-down effects of macroconsumers on ER via predation on shredding macroinvertebrates. The high rates of prey exchange between experimental patches and the rest of the stream reaches make definitive assessments of macroconsumer predation effects on our one-time macroinvertebrate samples challenging. Despite the limitations of patch-based exclusion for detecting top-down effects of macroconsumers, other approaches also include inherent challenges, and high flows at our experimental sites made larger-scale reach removals of consumers logistically difficult.

Understanding the role of animals in ecosystem processes, and in carbon cycling in particular, has been highlighted as an important knowledge gap (Schmitz et al. 2014). While our experimental design failed to detect net effects of our stream macroconsumers on ecosystem rates or the basal resource pools that drive them, we identified bottom-up, basal resource drivers of both ER and GPP. Our approach of removing the community of large macroconsumers rather than individual species or functional groups simulates a potential worst-case scenario for macroconsumer community change, but may have obscured more subtle species- or trophic-

group specific effects on our measured standing stocks and processes. Future research targeting specific trophic groups for removal over longer time periods may help resolve the role of macroconsumers in carbon and nutrient cycling in streams.

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Table 5.1 Sampled stream networks with estimated biomass of macroconsumers in each reach at the end of experimental exclusion. Location indicates relative location of reach within the sampled network (Down = most downstream; Up = most upstream; Mid = mid-network). Total biomass is sum of macroconsumer functional groups per streambed area. Algivore, omnivore, and insectivore are areal biomass of each respective trophic group; values in parentheses are proportion of total biomass for each trophic group. Network abbreviations are as in Fig. 5.2. Note that two experimental reaches each in CPC and ARC had no macroconsumers present at the end of the exclusion and are not included in this table.

Network	Biome	Time	Network location	Total ($g\ m^{-2}$)	Algivore ($g\ m^{-2}$)	Omnivore ($g\ m^{-2}$)	Insectivore ($g\ m^{-2}$)	Dominant macroconsumers
LUQ	Tropical forest	Feb. – Mar. 2013	Down	4.24	0.06 (0.01)	3.27 (0.77)	0.91 (0.21)	*Atya* spp. (roughback shrimp), *Sicydium plumieri* (Sirajo goby), *Agonostomus monticola* (mountain mullet), *Xiphocaris elongate* (yellow-nosed shrimp)
			Mid	5.63	0.06 (0.01)	3.92 (0.70)	1.65 (0.29)	*Atya* spp. (roughback shrimp), *Sicydium plumieri* (Sirajo goby), *Agonostomus monticola* (mountain mullet), *Xiphocaris elongate* (yellow-nosed shrimp)
			Up	2.01	-	2.01 (1.0)	-	*Atya* spp. (roughback shrimp), *Xiphocaris elongate* (yellow-nosed shrimp)
CWT	Deciduous forest	Mar. – Apr. 2013	Down	2.48	-	0.64 (0.26)	1.83 (0.74)	*Cambarus bartonii* (Appalachian brook crayfish), *Cottus bairdii* (mottled sculpin), *Rhinichthys cataractae* (longnose dace), *Salvelinus fontinalis* (brook trout),
			Mid	0.83	-	0.59 (0.71)	0.24 (0.29)	*Cambarus bartonii* (Appalachian brook crayfish), *Desmognathus quadramaculatus* (black belly salamander), *Salvelinus fontinalis* (brook trout)
			Up	2.04	-	0.74 (0.36)	1.30 (0.64)	*Cambarus bartonii* (Appalachian brook crayfish), *Desmognathus quadramaculatus* (black belly salamander), *Eurycea wilderae* (Blue Ridge two-lined salamander)

KNZ	Tallgrass prairie	May – Jun. 2013	Down	6.49	4.28 (0.66)	0.63 (0.10)	1.58 (0.24)	*Campostoma anomalum* (central stoneroller), *Etheostoma spectabile* (Orangethroat darter), *Noturus exilis* (slender madtom), *Orconectes* spp. (crayfish), *Phoxinus erythrogaster* (Southern redbelly dace), *Semotilus atromaculatus* (creek chub)
			Up	0.54	0.31 (0.57)	0.12 (0.22)	0.11 (0.20)	*Campostoma anomalum* (central stoneroller), *Etheostoma spectabile* (Orangethroat darter), *Orconectes* spp. (crayfish), *Phoxinus erythrogaster* (Southern redbelly dace)
CPC	Boreal forest	Jul. – Aug. 2013	Down	6.45	-	-	6.45 (1.00)	*Cottus cognatus* (slimy sculpin), *Oncorhynchus mykiss* (rainbow trout), *Thymallus arcticus* (Arctic grayling)
ARC	Tundra	Jul. – Aug. 2013	Mid	0.07	-	-	0.07 (1.00)	*Thymallus arcticus* (Arctic grayling)

Table 5.2 Linear mixed effects models and parameter estimates for relationships between chamber rates (ecosystem respiration [ER; mg O_2 m^{-2} min^{-1}], gross primary production [GPP; mg O_2 m^{-2} min^{-1}]; ammonium [NH_4] uptake [µg N m^{-2} min^{-1}]) and hypothesized basal resource drivers (coarse and fine benthic organic matter [CBOM, FBOM; g AFDM m^{-2}], chlorophyll-a [chl-a; mg m^{-2}]) from consumer treatment (exclusion vs. control) patches in three streams in each of five biomes. Model selection was based on AIC_C values. All models for ER and GPP included a nested random effect of stream within biome to account for the hierarchical experimental design. Hypothesized drivers and rates (except NH_4 uptake) were natural log (x) or natural log (x + 1) transformed to meet assumptions of normality. The number of parameters (K), AIC_C score, change in AIC_C from the top model (ΔAIC_C), weight and cumulative weight of support (W_i, $CumW_i$), log likelihood (LL), and conditional R^2 (R_c^2) are reported for models where $\Delta AIC_C \leq 2$ and are ranked by increasing AIC_C value, along with degrees of freedom (df), F statistics (F) and P-values. P-values in bold indicate statistical significance ($\alpha = 0.05$).

Model	**K**	**AIC_C**	**ΔAIC_C**	**W_i**	**$CumW_i$**	**LL**	**R_c^2**	**df**	**F**	**P**
Ecosystem respiration										
Biome	8	375.50	0	0.24	0.24	-179.39	0.40			
Intercept								1, 179	244.43	**<0.001**
Biome								4, 9	33.51	**<0.001**
CBOM + Biome + Treatment	10	376.00	0.50	0.19	0.43	-178.60	0.41			
Intercept								1, 176	234.16	**<0.001**
ln (CBOM + 1)								1, 176	39.31	**<0.001**
Biome								4, 9	22.38	**<0.001**
Treatment								1, 13	2.27	0.156
CBOM + Biome	9	376.12	0.63	0.18	0.74	-178.93	0.40			
Intercept								1, 176	236.13	**< 0.001**
ln (CBOM + 1)								1, 176	40.04	**< 0.001**
Biome								4, 9	22.51	**< 0.001**
FBOM + Biome	9	376.78	1.28	0.13	0.74	-178.93	0.40			
Intercept								1, 177	239.59	**<0.001**
ln (FBOM + 1)								1, 177	80.26	**<0.001**
Biome								4, 9	13.02	**0.001**

FBOM + Biome + Treatment	10	376.80	1.30	0.13	0.87	-177.84	0.41			
Intercept								1, 177	237.59	< **0.001**
ln (FBOM + 1)								1, 177	79.40	< **0.001**
Biome								4, 9	12.93	**0.001**
Treatment								1, 13	2.13	0.169
Gross primary production										
Chl-a + Biome + Treatment	10	434.91	0.00	0.42	0.42	-206.89	0.32			
Intercept								1, 175	158.91	<**0.001**
ln (Chl-a + 1)								1, 175	45.60	<**0.001**
Biome								4, 9	8.72	**0.004**
Treatment								1, 13	2.31	0.153
Chl-a + Biome	9	435.00	0.08	0.40	0.83	-208.03	0.32			
Intercept								1, 175	157.75	<**0.001**
ln (Chl-a + 1)								1, 175	45.09	<**0.001**
Biome								4, 9	8.67	**0.004**
Ammonium uptake										
Intercept only	4	-27.39	0	0.46	0.46	17.82	-			
Intercept								1, 140	52.50	<**0.001**

Table 5.3 Summary of rates and standing stocks in patch-scale measurements. Exclusion and treatment patches were pooled because they did not significantly differ. Value are mean ± 1 SE of patches across 3 streams within each network.

Stream network	ER (mg O_2 m^{-2} min^{-1})	GPP (mg O_2 m^{-2} min^{-1})	NEP (mg O_2 m^{-2} min^{-1})	NH4 uptake (µg m^{-2} sec^{-1})	CBOM (g AFDM m^{-2})	FBOM (g AFDM m^{-2})	Chl-a (mg AFDM m^{-2})
LUQ	0.04 ± 0.01	0.02 ± 0.01	-0.02 ± 0.00	0.42 ± 0.04	14.1 ± 3.5	6. 8 ± 0.9	3.4 ± 1.4
CWT	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	NA	19.6 ± 2.8	13.4 ± 2.1	2.6 ± 0.3
KNZ	1.86 ± 1.68	1.82 ± 1.68	-0.04 ± 0.02	0.47 ± 0.10	22.5 ± 3.8	881.1 ± 211.5	65.0 ± 6.1
CPC	0.43 ± 0.12	0.27 ± 0.12	-0.15 ± 0.01	0.46 ± 0.04	126.1 ± 14.1	365.3 ± 30. 9	8.8 ± 1.1
ARC	0.22 ± 0.12	0.17 ± 0.12	-0.04 ± 0.00	0.21 ± 0.05	14.4 ± 1.9	6.3 ± 0.9	13.4 ± 1.7

Figure Legends

Fig. 5.1 Conceptual diagram of predicted macroconsumer effects on ecosystem structure and function for (A) gross primary production and (B) ecosystem respiration. Solid lines and arrows indicate potential direct consumptive effects while dashed lines and arrows indicate potential indirect consumptive effects. Plus (+) indicates predicted positive relationship; minus (-) indicates predicted negative relationship.

Fig. 5.2 Map of SCALER experimental sites, with corresponding terrestrial biome and site latitude and longitude. Note that areas outside the contiguous United States are not to scale, but are sized to show location of sampled watershed.

Fig. 5.3 Cross-biome basal resource drivers of ecosystem rates measured in chambers. Ecosystem respiration (ER) was positively correlated with both (A) coarse and (B) fine benthic organic matter in patch baskets, while gross primary production (GPP) was positively correlated with (C) chlorophyll-a (chl-a). Shapes correspond to SCALER biomes (LUQ: open circle; CWT: filled circle; KNZ: open triangle up; CPC: filled triangle up; ARC: open triangle down) and indicate mean values for each sampled stream ± 1 SE. Note that all axes are natural log (x + 1) transformed. Ranges of site means of back transformed logged values are: ER (0.14 – 5.67 mg O_2 m^{-2} min^{-1}), GPP (0.08 – 7.98 mg O_2 m^{-2} min^{-1}), CBOM (6.75 - 142 g AFDM m^{-2}), FBOM (2.60 – 1807 g AFDM m^{-2}), chl-a (0.37 – 81.40 mg m^{-2}). All rates also had a significant effect of biome, but no biome × basal resource interaction. Lines indicate significant ($\alpha = 0.05$) fits from one or more top linear mixed effects models ($\Delta AIC_c \leq 2$; Table 5.2).

Fig. 5.4 Response ratios showing effects of macroconsumer exclusion on (A) ecosystem respiration (ER), (B) gross primary production (GPP), and (C) ammonium (NH_4) uptake. Results are shown as natural log response ratios ± 95% confidence intervals of paired control over exclusion patches. Bars within each biome indicate network position (white: downstream; gray: mid-network; black: upstream). ND = no data available.

Fig. 5.5 Cross-biome relationships between macroinvertebrates and their primary food sources: (A) shredders and coarse benthic organic matter (CBOM), (B) collector-gatherers and fine benthic organic matter (FBOM), (C) scrapers and chlorophyll-a (chl-a), (D) Predators and total macroinvertebrate biomass. Shapes correspond to SCALER biomes (LUQ: open circle; CWT: filled circle; KNZ: open triangle up; CPC: filled triangle up; ARC: open triangle down) and indicate mean values for each sampled stream ± 1 SE. Solid line indicates significant ($\alpha = 0.05$) cross-biome effect. Note that all axes are natural log (x + 1) transformed. Ranges of back transformed values are: CBOM (6.75 – 142 g AFDM m^{-2}), FBOM (2.60 – 1807 g AFDM m^{-2}), chl-a (0.37 – 81.40 mg m^{-2}), shredder biomass (0 – 824 mg m^{-2}), collector biomass (6.38 – 21341 mg m^{-2}), scraper biomass (1.90 – 1463 mg m^{-2}), predator biomass (7.67 – 1146 mg m^{-2}).

Fig. 5.6 Cross-biome relationships between macroconsumers and their primary food sources: (A) omnivores and coarse benthic organic matter (CBOM), (B) omnivores and fine benthic organic matter (FBOM), (C) algivores and chlorophyll-a (chl-a), (D) Invertivores (omnivores + insectivores) and total macroinvertebrate biomass. Shapes correspond to SCALER biomes (LUQ: open circle; CWT: filled circle; KNZ: open triangle up; CPC: filled triangle up; ARC: open triangle down) and indicate mean values for each sampled stream ± 1 SE. Solid line

indicates significant ($\alpha = 0.05$) cross-biome effect; dashed line indicates marginally significant ($\alpha = 0.10$) effect. Note that all axes are natural log (x + 1) transformed. Ranges of back transformed values are: CBOM (6.75 – 142 g AFDM m^{-2}), FBOM (2.60 – 1807 g AFDM m^{-2}), chl-a (0.37 – 81.40 mg m^{-2}), omnivore biomass (0 – 3.92 g m^{-2}), algivore biomass (0 – 4.28 g m^{-2}), and insectivore biomass (0 – 6.45 g m^{-2}).

Fig. 5.1

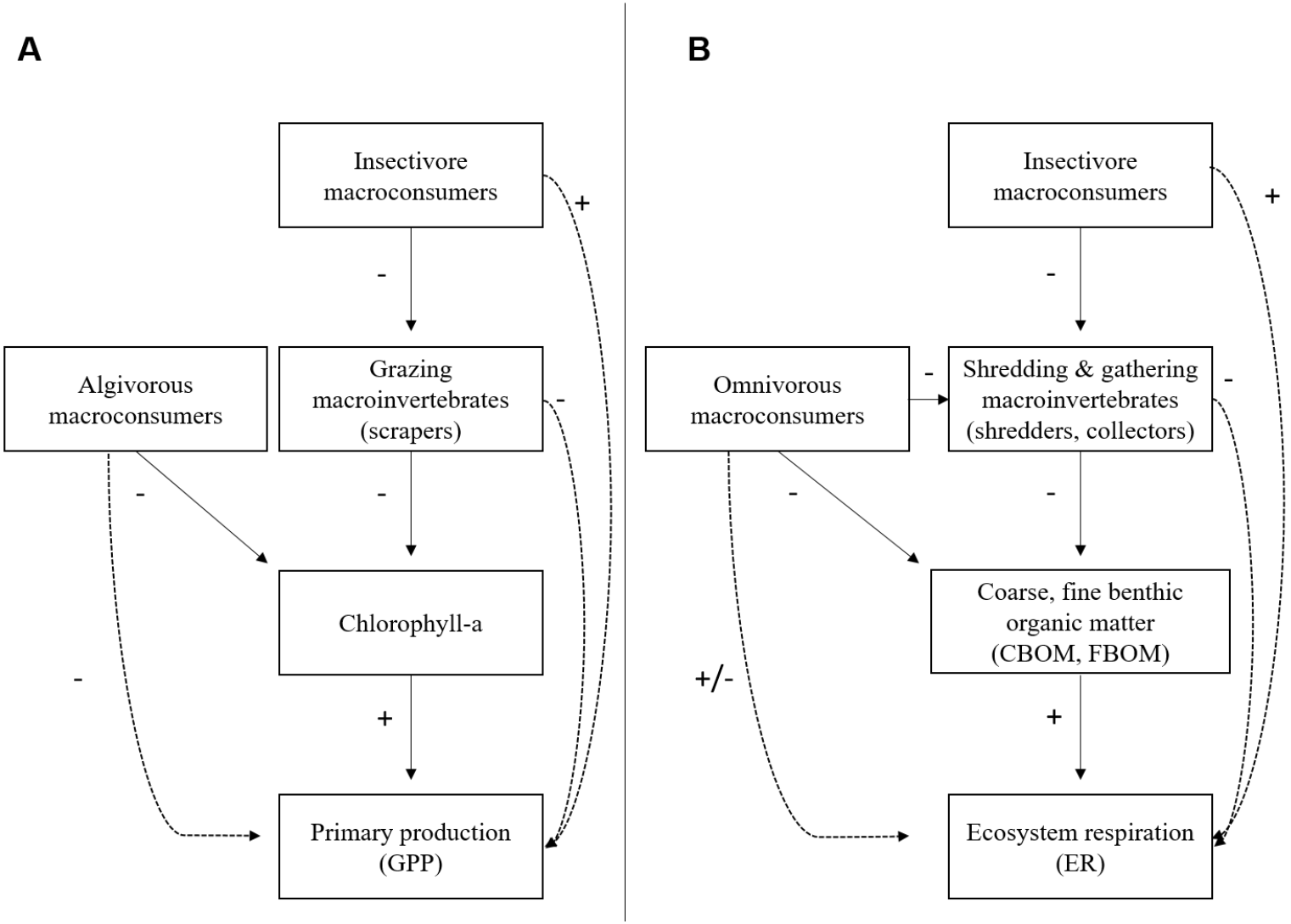

A
Insectivore macroconsumers
+
-
Algivorous macroconsumers
Grazing macroinvertebrates (scrapers)
-
-
-
Chlorophyll-a
-
+
Primary production (GPP)
B
Insectivore macroconsumers
+
-
Omnivorous macroconsumers
-
Shredding & gathering macroinvertebrates (shredders, collectors)
-
-
-
Coarse, fine benthic organic matter (CBOM, FBOM)
+/-
+
Ecosystem respiration (ER)

Fig. 5.2

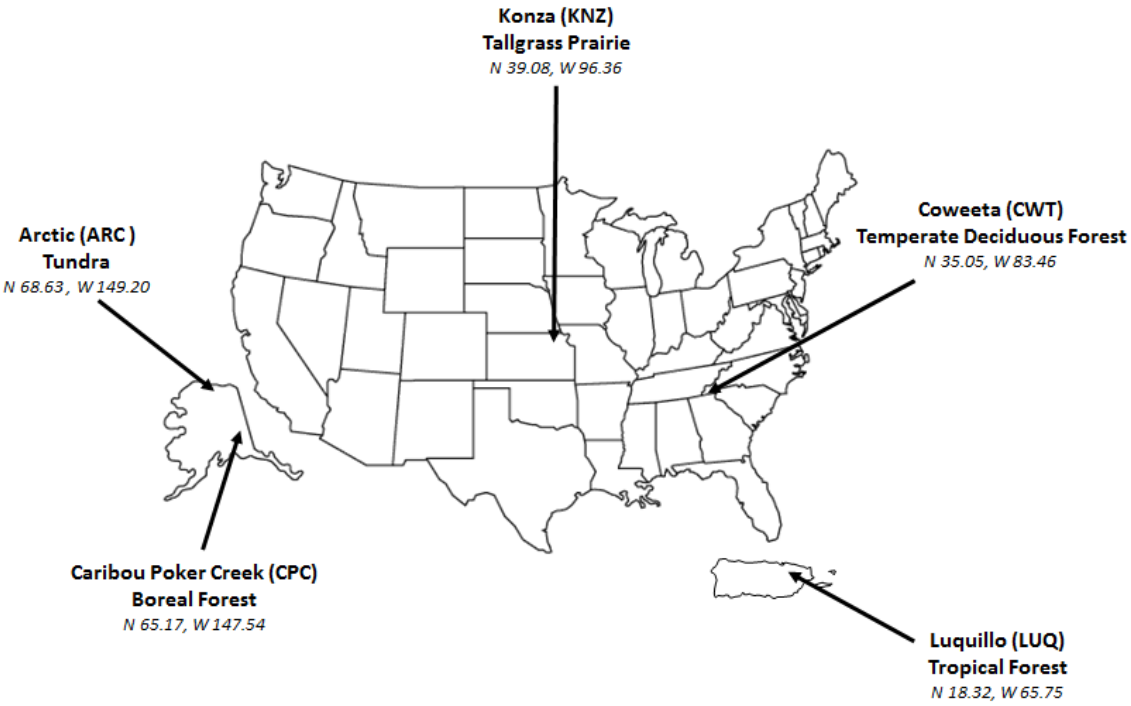

Konza (KNZ)
Tallgrass Prairie
N 39.08, W 96.36
Arctic (ARC)
Tundra
N 68.63, W 149.20
Coweeta (CWT)
Temperate Deciduous Forest
N 35.05, W 83.46
Caribou Poker Creek (CPC)
Boreal Forest
N 65.17, W 147.54
Luquillo (LUQ)
Tropical Forest
N 18.32, W 65.75

Fig. 5.3

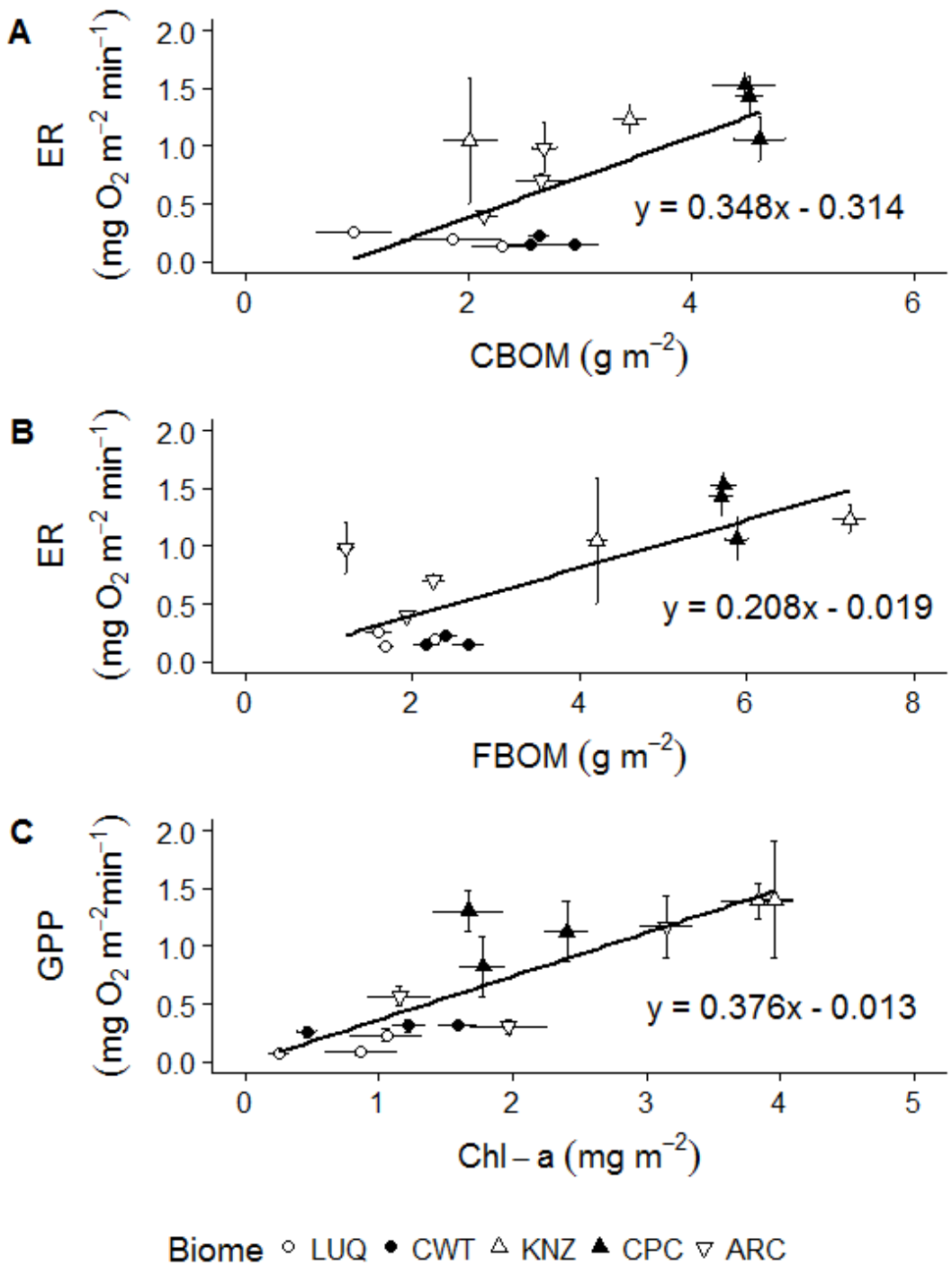

A
ER
(mg O2 m-2 min-1)
y = 0.348x - 0.314
CBOM (g m-2)
B
ER
(mg O2 m-2 min-1)
y = 0.208x - 0.019
FBOM (g m-2)
C
GPP
(mg O2 m-2min-1)
y = 0.376x - 0.013
Chl – a (mg m-2)
Biome ○ LUQ ● CWT △ KNZ ▲ CPC ▽ ARC

Fig. 5.4

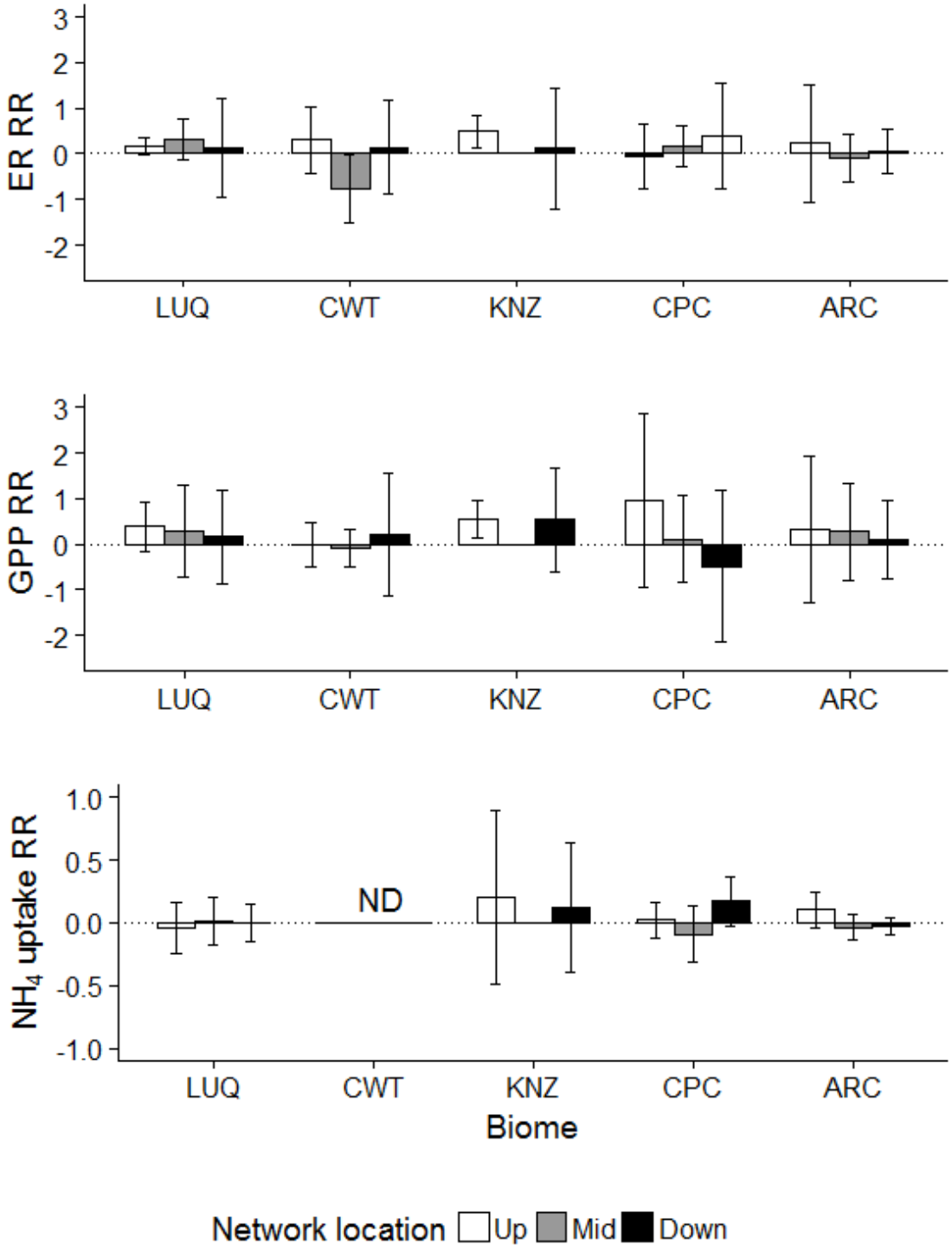

ER RR
GPP RR
NH_4 uptake RR
ND
LUQ
CWT
KNZ
CPC
ARC
Biome
Network location
Up
Mid
Down

Fig. 5.5

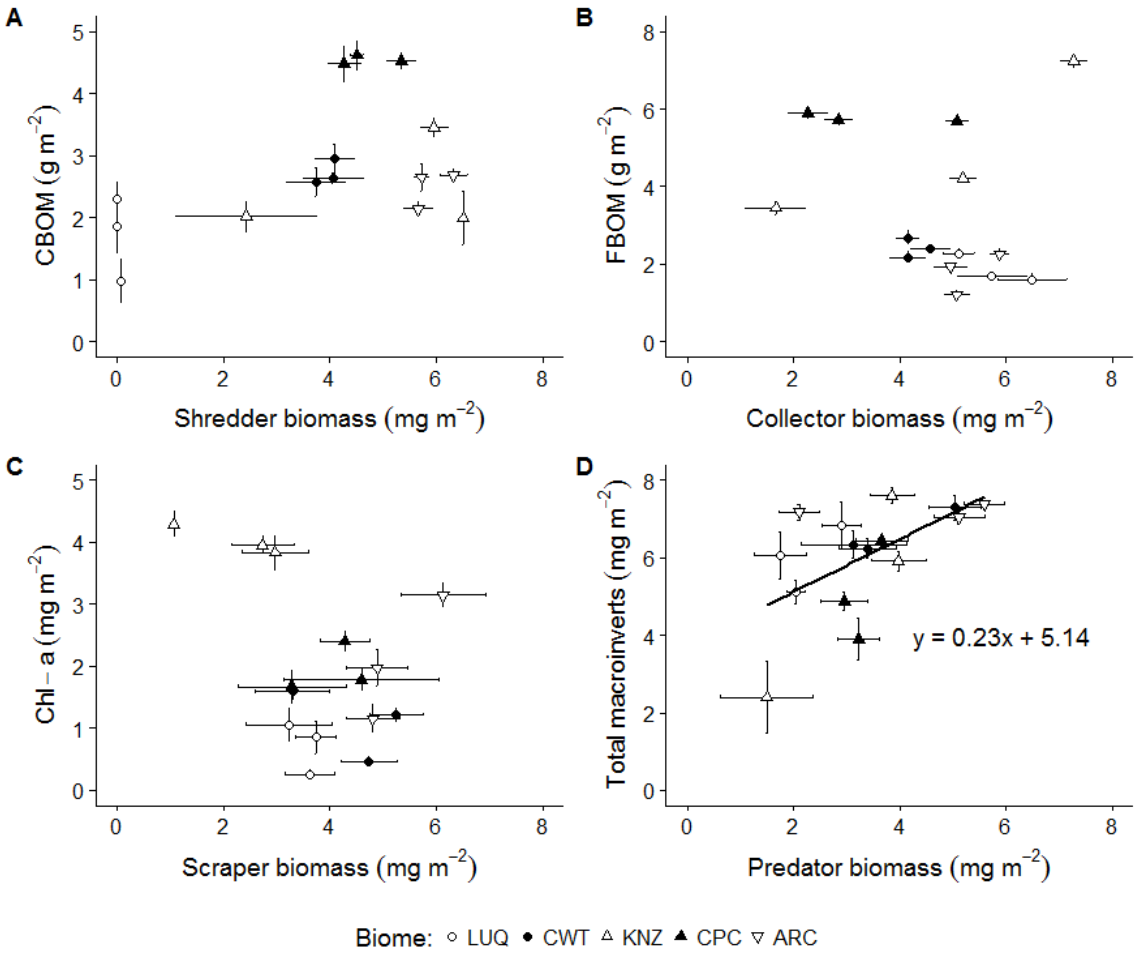
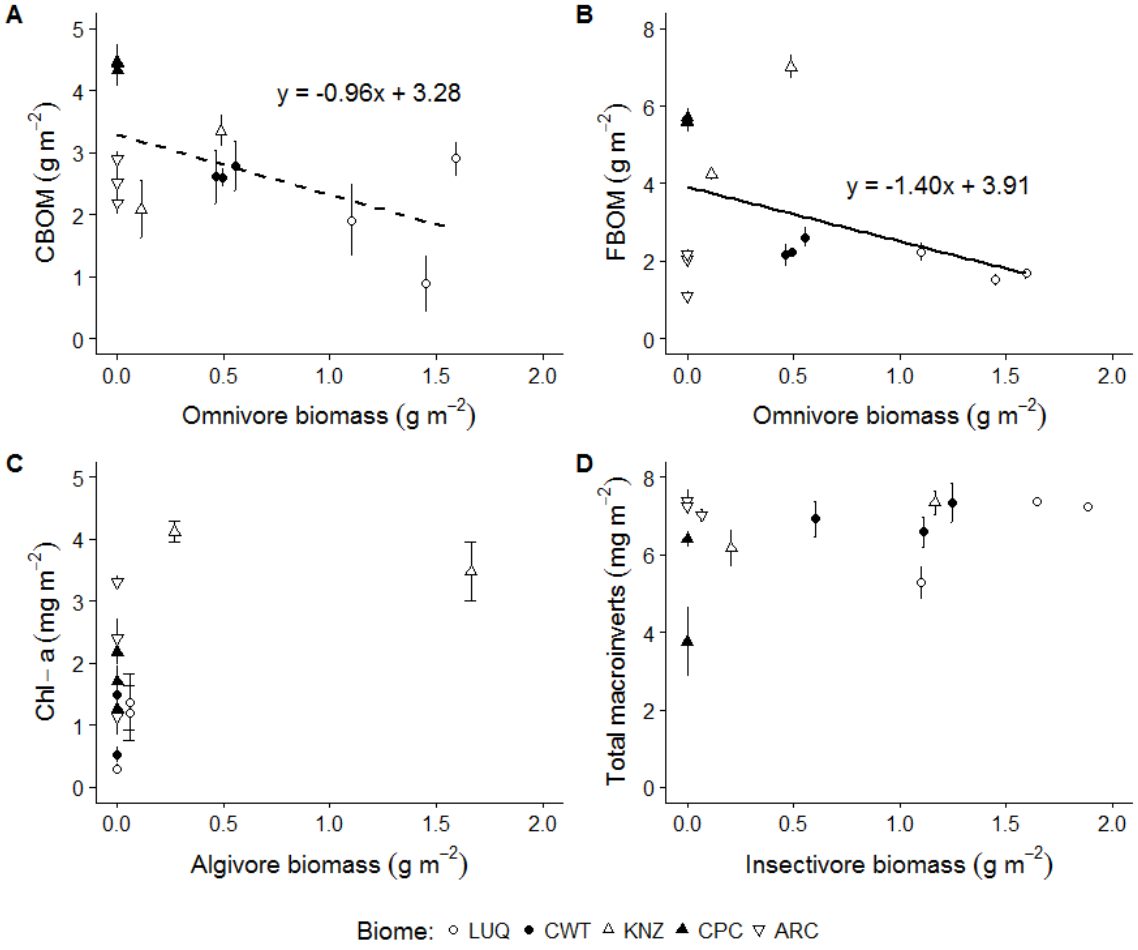

A
CBOM (g m^{-2})
Shredder biomass (mg m^{-2})
B
FBOM (g m^{-2})
Collector biomass (mg m^{-2})
C
Chl – a (mg m^{-2})
Scraper biomass (mg m^{-2})
D
Total macroinverts (mg m^{-2})
Predator biomass (mg m^{-2})
y = 0.23x + 5.14
Biome: ○ LUQ ● CWT △ KNZ ▲ CPC ▽ ARC

Fig. 5.6


A
y = -0.96x + 3.28
CBOM (g m^{-2})
Omnivore biomass (g m^{-2})
B
y = -1.40x + 3.91
FBOM (g m^{-2})
Omnivore biomass (g m^{-2})
C
Chl – a (mg m^{-2})
Algivore biomass (g m^{-2})
D
Total macroinverts (mg m^{-2})
Insectivore biomass (g m^{-2})
Biome: ○ LUQ ● CWT △ KNZ ▲ CPC ▽ ARC

CHAPTER 6:

CONCLUSIONS

Headwater streams, which form the smallest branches of river networks, provide valuable services to society, including the maintenance of aquatic biodiversity (Meyer et al. 2007), improved water quality through nutrient retention (Webster 2007), and aesthetic and recreational benefits (Dudgeon et al. 2006). Despite these services and the dominance of headwater streams by channel length in river networks (Leopold et al. 1964, Benstead and Leigh 2012), headwater streams are subject to degradation and alteration through land use changes (Meyer and Wallace 2001), nutrient pollution, climate changes, and compromised connectivity .

Given the importance of headwater streams and the currently tenuous status of their ongoing protection and preservation (Palmer et al. 2010, Hawkins 2015), it is essential that stream research take a network perspective to understand the generality of patterns and processes measured in single reaches (Fisher 1997, Meyer and Wallace 2001). In addition, understanding how the physical and biological drivers of ecosystem rates, and in turn ecosystem functions, differ throughout stream networks is an essential step toward predicting how functions in stream networks may change with future alterations due to riparian land use and climate change.

In this dissertation, I have leveraged my participation in the collaborative MacroSystems Biology “Scale, Consumers, and Lotic Ecosystem Rates” (SCALER) project to quantify aspects of stream structure and function both within a headwater stream network using experimental measurements and synoptic surveys, and among distinct headwater stream networks from across North America to understand patterns and processes in streams more broadly. In Chapters 2 and

3, I addressed the role of basal resources as drivers of ecosystem rates within the Coweeta network, and compared the quality of basal resources across SCALER stream networks using a stoichiometric approach. Chapters 4 and 5 focused on more explicit tests of the role of macroconsumers on ecosystem rates, to determine whether the consumers in Coweeta headwater streams (Chapter 4), and across SCALER streams (Chapter 5) initiated trophic cascades and/or alleviated nutrient demand via excretion.

In Chapter 2, we determined that recirculating chambers can provide insights about stream functions by isolating biological drivers from the hydrologic drivers present in reach-scale measurements. We found that rates of chamber respiration (ER) and gross primary production (GPP) were driven by benthic organic matter standing stocks and light availability, respectively, and that drivers of both rates varied substantially between seasons, leading to higher ER in September and higher GPP in April. When we scaled up chamber measurements to the reach scale using point measurements of stream temperature and light, we found that there were larger differences between season than across the network within a given season. Network position affected gross primary production (GPP), which increased with distance downstream during early fall, when the canopy was closed prior to leaf-fall. Using our scaled rates and estimates of the wetted area of different sized streams in the headwaters of the Little Tennessee River basin, we estimated that 1^{st} and 2^{nd} order streams play a substantial role in total ER and ammonium uptake compared to 3^{rd} and 4^{th} order streams, contributing 76% and 69% of estimated function in the 1^{st} – 4^{th} order portion of the network, respectively. In addition, despite heavy shading, 1^{st} and 2^{nd} order streams were responsible for 50% of GPP in the 1^{st} – 4^{th} order network. These findings collectively emphasize the importance of understanding drivers of

ecosystem rates, and that variation in ecosystem rates, even across relatively small spatial extents, can have substantial effects for the functioning of stream networks.

In Chapter 3, we compared the quality of two pools of detrital basal resources, by quantifying the stoichiometric (carbon to nitrogen [C:N] and phosphorus [C:P]) ratios for coarse and fine benthic organic matter across four stream networks within the SCALER project. We found that across biomes, coarse benthic organic matter (CBOM) was of lower quality (higher C:N and C:P), and was more variable in those stoichiometric ratios, than fine benthic organic matter (FBOM). While the nutrient content and stoichiometry of CBOM likely reflected the varying quality of allochthonous inputs among sampled stream networks, the low variability of FBOM stoichiometric ratios suggests that in-stream microbial breakdown of recalcitrant carbon homogenizes the detrital resource pool. The distinct stoichiometry of CBOM versus FBOM could have consequences for macroinvertebrate detritivores that feed on CBOM (shredders) and FBOM (collectors). Measured CBOM C:N and C:P across all four stream networks frequently exceeded estimates of shredder threshold elemental ratios (TER), which suggests that those consumers may often face nutrient (N and/or P) rather than carbon limitation. In contrast, most FBOM samples had C:N and C:P below collector-gatherer TERs, which suggests they may be more likely carbon than nutrient limited. Future investigations into the variability and flexibility of detritivore TERs will improve our understanding of the role of consumer-resource stoichiometric imbalances on carbon and nitrogen cycling in streams.

In Chapter 4, we manipulated the biomass of larval *Desmognathus quadramaculatus* salamanders using an enclosure experiment in a headwater stream in Coweeta, with the goal of understanding whether salamanders initiate trophic cascades in streams. We had predicted that salamander biomass would be negatively correlated with macroinvertebrate biomass, and that

reductions in macroinvertebrate shredder biomass would lead to decreased rates of litter breakdown and increased rates of respiration and ammonium (NH_4) uptake. Instead, we were unable to detect top-down effects of salamanders on our metrics of ecosystem structure and function. Rates of litter breakdown were similar to previously measured breakdown rates across Coweeta, and were not related to either leaf pack shredder biomass nor salamander biomass. However, we did detect an effect of salamanders on NH_4 uptake, as salamander biomass was inversely correlated with uptake rate. This suggests that salamanders may affect ecosystem rates more through bottom-up excretion of limiting nutrients than through top-down predation. However, our replication of measurements of respiration and NH_4 uptake was reduced due to a heavy rain that toppled enclosures, and future studies are needed to more precisely quantify the biomass-specific role of larval salamanders on metrics of stream structure and function.

In Chapter 5, I synthesized the effects of macroconsumers and macroinvertebrates on their primary food resources across the five SCALER headwater stream networks from distinct biomes, with the goal of determining whether there are broad patterns in top-down control in stream networks. In each headwater network, we used paired consumer exclusion and control patches and recirculating chambers to quantify rates of respiration (ER), gross primary production (GPP), and NH_4 uptake, and standing stocks of benthic organic matter and chlorophyll-a. When we compared basal resource standing stocks with the biomass of macroinvertebrates and macroconsumers that feed on them, we did not find strong support for consumptive effects of consumers. However, across sampled biomes, basal resources were significant drivers of ecosystem rates; benthic organic matter quantity was positively correlated with ER, while GPP increased with increasing chlorophyll-a concentrations. This suggests that

across diverse stream networks, bottom-up resource control of rates was a stronger driver than top-down consumptive effects of consumers.

Understanding the roles of basal resources and macroconsumers in driving stream functions is essential, given ongoing pressure from land use and climate changes. The quantity and quality of basal resources entering streams from the riparian area can change when forests are converted to agricultural or urban land uses (Walsh et al. 2005, Milanovich et al. 2014), while nutrient runoff into streams can further alter detrital stoichiometry (Scott et al. 2013), decreasing C:N and C:P and accelerating breakdown by stream macroinvertebrates and microbes (Manning et al. 2015). Increased detrital loss rates and reduced standing stocks in turn affect carbon export from streams (Rosemond et al. 2015), with broad implications for carbon cycling, as lotic freshwater ecosystems are increasingly recognized for their role in the transport, processing, and emission of terrestrially-sourced carbon (Cole et al. 2007, Raymond et al. 2013, Hotchkiss et al. 2015). In addition, increased temperatures and altered precipitation patterns as a result of climate change are predicted to further accelerate detrital loss, as breakdown rates accelerate and episodic high flows scour detritus from streams (Kominoski and Rosemond 2012 and references therein). Quantifying the role of basal resources in relatively undisturbed headwater streams, and comparing relationships across diverse stream networks, can provide a baseline understanding against which we can predict how land use and climate change may change stream functions.

Aquatic macroconsumers are also subject to threats from land use and climate change. The community composition of stream organisms has already been altered by human-induced changes in flow regime, nutrient loading, and habitat degradation (Allan 2004, Dudgeon et al. 2006, Woodward et al. 2010). Ongoing climate changes are expected to exacerbate stressors on

stream consumers, and increase the frequency of species loss (Ricciardi and Rasmussen 1999, Perkins et al. 2010, Woodward et al. 2010). Stream warming is expected to reduce the habitat availability, and thus range and long-term viability of many stream consumers, including species of fishes (e.g., Isaak and Rieman 2013), salamanders (Milanovich et al. 2010), and macroinvertebrates (Palmer et al. 2009 and references therin). Given these pressures, understanding the roles of stream consumers, through both top-down pathways of consumption and bottom-up pathways of nutrient storage and regeneration, is important for predicting how changes in consumer abundance and community composition may alter stream ecosystem functions.

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Appendix A

Table A1 Chamber rates of ecosystem respiration (ER), gross primary production (GPP), and net ecosystem production (NEP) in April 2013 (E1 – E3) and September 2014 (all sites). Light availability during NEP (PAR) and water temperature (Temp.) are values within chambers during rate measurements. Coarse (CBOM) and fine (FBOM) benthic organic matter and chlorophyll-a (chl-a) were quantified from baskets in each replicate set not run in the chamber. All values are mean ± 1 SE.

ID	Season	ER (mg O_2 m^{-2} min^{-1})	GPP (mg O_2 m^{-2} min^{-1})	NEP (mg O_2 m^{-2} min^{-1})	PAR (µmol γ m^{-2} sec^{-1})	Temp. (°C)	CBOM (g AFDM m^{-2})	FBOM (g AFDM m^{-2})	Chl-a (mg AFDM m^{-2})
E1	April	0.41 ± 0.13	0.95 ± 0.31	0.54 ± 0.18	228.2 ± 19.0	8.66 ± 0.07	26.0 ± 10.3	16.2 ± 5.0	1.01 ± 0.41
	Sept.	0.53 ± 0.28	0.16 ± 0.13	-0.36 ± 0.15	11.87 ± 0.27	17.51 ± 0.00	122.4 ± 70.6	105.4 ± 46.8	0.16 ± 0.02
E2	April	0.19 ± 0.10	0.94 ± 0.10	0.76 ± 0.16	76.37 ± 4.64	8.62 ± 0.06	22.1 ± 10.6	9.6 ± 2.7	4.00 ± 1.19
	Sept.	0.32 ± 0.07	0.11 ± 0.03	-0.21 ± 0.05	12.63 ± 0.51	17.40 ± 0.00	107.1 ± 28.7	82.2 ± 33.7	0.49 ± 0.08
E3	April	0.38 ± 0.08	0.56 ± 0.18	0.18 ± 0.13	23.42 ± 1.05	13.61 ± 0.03	13.4 ± 2.0	8.7 ± 0.7	2.05 ± 0.56
	Sept.	0.37 ± 0.07	0.08 ± 0.03	-0.29 ± 0.05	15.58 ± 1.34	18.62 ± 0.00	282.9 ± 120.1	107.1 ± 12.0	0.26 ± 0.21
S5	Sept.	0.47 ± 0.07	0.08 ± 0.03	-0.39 ± 0.07	15.46 ± 0.40	18.72 ± 0.01	99.2 ± 21.7	96.2 ± 16.8	0.66 ± 0.21
S9	Sept.	0.31 ± 0.03	0.05 ± 0.03	-0.26 ± 0.03	2.43± 0.06	17.69 ± 0.00	64.7 ± 22.0	119.5 ± 14.5	0.22 ± 0.05
S12	Sept.	0.32 ± 0.03	0.03 ± 0.02	-0.29 ± 0.03	4.00 ± 0.08	17.64 ± 0.00	113.6 ± 44.0	167.0 ± 28.0	--
S16	Sept.	0.38 ± 0.04	0.07 ± 0.02	-0.30 ± 0.03	11.03 ± 0.52	18.47 ± 0.01	100.6 ± 17.4	163.4 ± 26.1	0.32 ± 0.08
S17	Sept.	0.66 ± 0.40	0.15 ± 0.13	-0.51 ± 0.26	2.73± 0.09	17.19 ± 0.00	156.6 ± 33.1	155.6 ± 30.8	0.16 ± 0.07
S19	Sept.	1.17 ± 0.60	0.90 ± 0.60	-0.27 ± 0.05	9.39 ± 0.26	14.38 ± 0.01	213.4 ± 40.5	284.0 ± 52.3	0.27 ± 0.14

Appendix B

Table B1. Biomass and abundance of select macroinvertebrate functional feeding groups identified from leaf packs on day 63. Range of salamander biomass within each treatment (n = 3 enclosures) on day 63 is indicated in parentheses. Within each salamander treatment, B and A indicate mean ± 1 SE of biomass (mg dry mass) or abundance (individuals), respectively. Functional feeding groups shown are collector-gatherers (CG), predators (P), and shredders (Sh). Letters before taxa names indicate orders for macroinvertebrate Insecta; C = Coleoptera, D = Diptera, E = Ephemeroptera, O = Odonata, P = Plecoptera, T = Trichoptera.

		0 Dq (0 g m^{-2})		3 Dq (3.0–3.9 g m^{-2})		5 Dq (4.1–5.9 g m^{-2})		10 Dq (12.3–12.9 g m^{-2})		15 Dq (10.0–19.4 g m^{-2})	
FFG	**Taxon**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**
CG	C- Elmidae	1.95 ± 0.00	1.0 ± 0.0	0.23 ± 0.00	2.0 ± 0.0	-	-	0.18 ± 0.00	1.0 ± 0.0	0.46 ± 0.14	1.7 ± 0.3
	E- *Habrophlebia*	0.30 ± 0.00	2.0 ± 0.0	0.27 ± 0.18	2.5 ± 0.5	0.33 ± 0.19	3.7 ± 1.5	1.18 ± 0.22	4.0 ± 0.0	0.93 ± 0.34	7.5 ± 5.5
	D- Non-Tanypodinae	0.19 ± 0.14	13.7 ± 8.3	0.51 ± 0.26	18.3 ± 7.7	0.21 ± 0.02	14.0 ± 4.4	0.33 ± 0.14	11.3 ± 6.2	0.61 ± 0.36	29.7 ± 15.8
	Oligochaeta	0.04 ± 0.03	5.5 ± 4.5	0.33 ± 0.13	9.5 ± 4.5	0.52 ± 0.37	9.3 ± 0.3	0.20 ± 0.15	12.3 ± 9.9	0.36 ± 0.12	8.7 ± 1.8
	D- *Ormosia*	-	-	-	-	0.22 ± 0.00	1.0 ± 0.0	-	-	0.02 ± 0.00	1.0 ± 0.0
	D- *Pericoma*	-	-	0.003 ± 0.00	1.0 ± 0.0	0.06 ± 0.00	2.0 ± 0.0	0.003 ± 0.00	1.0 ± 0.0	-	-
	D- *Sciaridae*	-	-	0.08 ± 0.06	2.5 ± 1.5	0.04 ± 0.02	1.7 ± 0.3	-	-	0.05 ± 0.03	1.7 ± 0.7
P	P- *Beloneuria*	0.54 ± 0.00	1.0 ± 0.0	0.54 ± 0.00	1.0 ± 0.0	-	-	-	-	-	-
	D- *Bezzia*	0.48 ± 0.22	4.0 ± 1.0	0.67 ± 0.24	4.3 ± 1.8	1.03 ± 0.08	5.0 ± 1.0	0.46 ± 0.15	1.5 ± 0.5	1.11 ± 0.25	5.5 ± 0.5
	O- *Cordulegaster*	-	-	-	-	-	-	-	-	0.14 ± 0.00	1.0 ± 0.0

	D- *Dicranota*	-	-	-	-	-	-	0.04 ± 0.00	2.0 ± 0.0	0.02 ± 0.00	1.0 ± 0.0
	D- Empididae	0.03 ± 0.00	1.0 ± 0.0	0.04 ± 0.00	2.0 ± 0.0	0.06 ± 0.00	2.0 ± 0.0		-	0.04 ± 0.00	2.0 ± 0.0
	D- *Hexatoma*	-	-	1.31 ± 0.96	1.0 ± 0.0	0.18 ± 0.00	3.0 ± 0.0	0.35 ± 0.00	1.0 ± 0.0	-	-
	P- *Isoperla*	0.87 ± 0.74	1.0 ± 0.0	-	-	1.73 ± 0.00	5.0 ± 0.0	-	-	-	-
	O- *Lanthus*	-	-	-	-	0.19 ± 0.00	1.0 ± 0.0	-	-	-	-
	D- *Pilaria*	0.02 ± 0.00	1.0 ± 0.0	-	-	-	-	-	-	0.26 ± 0.00	6.0 ± 0.0
	D- *Pseudolimnolphila*	-	-	-	-	-	-	0.06 ± 0.00	1.0 ± 0.0	-	-
	T- *Rhyacophila*	0.31 ± 0.00	1.0 ± 0.0	0.10 ± 0.05	1.0 ± 0.0	0.15 ± 0.00	1.0 ± 0.0	0.06 ± 0.00	1.0 ± 0.0	1.53 ± 0.00	3.0 ± 0.0
	P- *Sweltsa*	0.41 ± 0.00	2.0 ± 0.0	-	-	-	-	-	-	0.29 ± 0.00	2.0 ± 0.0
	D- Tanypodinae	0.10 ± 0.05	3.0 ± 0.0	0.03 ± 0.02	2.0 ± 0.6	0.05 ± 0.01	5.3 ± 2.8	0.02 ± 0.00	3.0 ± 0.0	0.10 ± 0.05	8.0 ± 3.2
Sh	C- *Anchytarsus*	-	-	6.35 ± 1.47	1.0 ± 0.0	4.29 ± 0.00	1.0 ± 0.0	0.50 ± 0.00	1.0 ± 0.0	12.95 ± 0.00	1.0 ± 0.0
	T- *Fattigia*	-	-	-	-	-	-	0.33 ± 0.00	1.0 ± 0.0	-	-
	T- *Lepidostoma*	0.22 ± 0.08	1.3 ± 0.3	0.15 ± 0.00	1.0 ± 0.0	1.14 ± 0.94	2.3 ± 0.7	1.93 ± 1.88	3.0 ± 2.0	1.15 ± 1.02	6.0 ± 3.6
	P- *Leuctra*	0.37 ± 0.30	4.7 ± 1.5	1.35 ± 0.63	4.3 ± 2.0	0.24 ± 0.18	1.7 ± 0.3	0.22 ± 0.00	1.0 ± 0.0	0.81 ± 0.46	11.3 ± 5.9
	D- *Molophilus*	-	-	-	-	-	-	1.47 ± 0.00	4.0 ± 0.0	0.22 ± 0.00	1.0 ± 0.0
	P- *Tallaperla*	4.14 ± 3.46	2.0 ± 1.0	0.03 ± 0.00	2.0 ± 0.0	0.70 ± 0.69	1.0 ± 0.0	-	-	2.48 ± 2.46	3.0 ± 2.0

Appendix C

Table C1 Methods of macroconsumer sampling and biomass estimation for SCALER sites. Field methods included electrofishing, seining, minnow traps, and/or angling, depending on target species and regulations within the stream network. Capture efficiency (*q*) was based on maximum likelihood (ML) or estimated (Est.) based on other reaches within the network based on the efficacy of depletion in each reach. Macroconsumer community biomass (N) was estimated based on maximum likelihood (ML) or the total biomass caught (Total catch). Values of *q* and N represent the range of estimates across sampled reaches.

Stream network	Methods (field)	Method (*q*)	Method (N)	q	N
LUQ	Electrofishing	ML	ML	0.3 – 0.6	114 - 1399
CWT	Electrofishing, seining	Est.	ML	0.2	271 - 1329
KNZ	Electrofishing, seining	ML	ML	0.3 – 0.5	33 - 3649
CPC	Electrofishing, minnow traps	Est.	Total catch	0.8	2610
ARC	Angling	Est.	Total catch	1.0	155