BIOTIC AND ABIOTIC EFFECTS ON BIOGEOCHEMICAL FLUXES ACROSS MULTIPLE SPATIAL SCALES IN A PRAIRIE STREAM NETWORK

by

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Abstract

Understanding the variability of ecological processes across spatial scales is a central issue in ecology, because increasing scale is often associated with increasing complexity. In streams, measurements of biogeochemical fluxes are important for determining ecosystem health and the downstream delivery of nutrients, but are often collected at scales with benthic areas measured in spatial areas from ~10 cm^2 to ~100 m^2 (referred to here as patch and reach, respectively), which are smaller than the scale that management decisions are made. Both biotic and abiotic factors will be important when attempting to predict (i.e. scale) biogeochemical rates, but few studies have simultaneously measured rates and their primary drivers at different spatial scales. In the first chapter, I used a conceptual scaling framework to evaluate the ability to additively scale biogeochemical rates by comparing measurements of ecosystem respiration (ER) and gross primary production (GPP) from patch to reach-scales across multiple sites over a two-year period in a prairie stream. Patch-scale measurements with and without fish (biotic factors) and abiotic factors measured simultaneously with metabolic rates suggest that abiotic conditions are stronger drivers of these rates. Patch-scale rates significantly overestimated reach rates for ER and GPP after corrections for habitat heterogeneity, temperature and light, and a variety of stream substrata compartments. I show the importance of determining abiotic and biotic drivers, which can be determined through observational or experimental measurements, when building models for scaling biogeochemical rates. In the second chapter, I further examined patch-scale abiotic and biotic drivers of multiple biogeochemical rates (ER, GPP, and ammonium uptake) using path analyses and data from chapter 2. Total model-explained variance was highest for ER (65% as R^2) and lowest for GPP and ammonium uptake (38%). Fish removal directly increased ammonium uptake, while all rates were indirectly affected by fish removal through changes in

either FBOM and /or algal biomass. Significant paths of abiotic factors varied with each model. Large-scale processes (i.e. climate change and direct anthropogenic disturbances), and local biotic and abiotic drivers should all be considered when attempting to predict stream biogeochemical fluxes at varying spatial scales.

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Chapter 1 - Introduction

Understanding the variability of ecological processes across spatial scales is a central issue in all ecology (Levin 1992), since increasing scale is often associated with increasing complexity and variability (Hewitt et al. 2007). Most management decisions are made at the ecosystem level (Duffy 2009), while field measurements are often made at smaller spatial scales. In streams, measurements are often collected at scales with benthic areas measured in spatial areas from ~10 cm^2 to ~100 m^2 (referred to here as patch and reach, respectively). Unidirectional flow is a unique characteristic of streams and adds more complexity to the concept of scale because organic matter, nutrients, and aquatic organisms can readily move (or cycle) at the watershed scale (e.g., Schlosser 1991, Fausch et al. 2002, Banner et al. 2009).

Understanding the underlying mechanisms and drivers of bottom-up scaling of biogeochemical rates will be vital for evaluating the direct and indirect effects of anthropogenic alterations to streams. Land-use change, loss of connectivity through dams and drought, and the myriad of effects anticipated from a changing climate will likely alter these processes in the future. In-stream nutrient uptake and other nutrient cycling processes dictate the downstream movement of nutrients, especially those derived from terrestrial runoff and ground water input (e.g., Peterson et al. 2001, Mulholland et al. 2008). Quantifying nutrient uptake, its functional relationship with nutrient concentrations, and its effect on downstream transport is important for assessing the effects of nutrient enrichment by anthropogenic sources (Bernot and Dodds 2005). Specifically, nitrogen delivery to coastal areas from agriculturally impacted watersheds can cause eutrophication, indirectly leading to hypoxic "dead zones" that impair water quality, fisheries, and ecosystem health (Diaz and Rosenburg 2008). In-stream metabolism is another fundamental ecosystem process since rates can be interpreted as indicators of biotic activity and

ecosystem structure (Dodds 2007) and are important to stream researchers as well as watershed managers. Stream metabolism can be used as a means of determining ecosystem health (Fellows et al. 2006) or as a means to evaluate ecosystem response to disturbance (Bunn et al. 1999).

In natural and disturbed systems, both biotic and abiotic factors impact stream biogeochemical rates at any given scale. These factors can vary significantly within a given stream reach, but are often associated with a specific stream habitat (i.e. riffles or pools; Pringle et al 1988, Vanni 2010). Understanding the primary abiotic and biotic drivers of the process of interest, and their distribution across the spatial area of interest will dictate the method utilized for scaling (i.e. additive, mechanistic, or, process based), and affect the accuracy of predicted rates at larger spatial scales.

Multiple concepts have been presented to help elucidate large-scale processes and patterns that occur across a stream network (i.e. from headwaters to large river basins). The first, and most notable was the River Continuum Concept (RCC), which describes how transportation of energy and carbon varies across stream networks (Vannote et al. 1980). The RCC has provided the basis for more context-dependent concepts including the flood pulse concept (Junk et al. 1989), the riverine ecosystem synthesis (Thorp et al. 2006), and the river wave concept (Humphries et al. 2014). These concepts have generated a useful basis for testing the effect of scale on ecosystem processes by suggesting the primary biotic and abiotic drivers present at each scale of interest; however, few studies have explicitly tested the effect of scale on ecosystem structure and function in streams.

The National Ecological Observatory network (NEON) will soon begin collecting observational data for at least 30 years across impacted and pristine streams in an attempt to empirically measure changes in aquatic ecosystems. Furthermore, the STReam Experimental and

Observatory Network (STREON) will experimentally manipulate larger animal (> ~1 cm) presence and in-stream nutrient concentrations over 10 of those years to better understand how the loss of larger aquatic animals and increasing nutrient concentrations affect these ecosystem processes. These initiatives will substantially improve our knowledge of future anthropogenic change in streams; however, interpreting this data may be difficult because of the scale with which it is measured. In particular, current plans to assess animal effects on ecosystem rates will be at the patch-scale, while biogeochemical rates and monitoring will happen at reach-scales (http://www.neoninc.org/our-design/collection-methods/streon).

Data for the research reported in this thesis was collected as a part of a larger project, SCALER (Scale Consumers And Lotic Ecosystem Rates), aiming to develop watershed, biome, and continental scale models of biogeochemical rates from nested measurements collected at small spatial scales. SCALER also seeks to determine the effect of fish removal on biogeochemical rates and the effect of fish removal on scaling rates to larger areas. The SCALER experiment is a precursor to STREON and the results of this thesis will offer valuable preliminary data to STREON experiments, which can be used to generate hypotheses for future studies with this initiative.

The major questions addressed in this thesis are: 1) Can we predict reach scale estimates of gross primary production (GPP) and ecosystem respiration (ER) with multiple measurements made at the patch scale and 2) what are the specific biotic and abiotic drivers of biogeochemical fluxes (GPP, ER, and ammonium uptake) at the patch scale. For the first data chapter I created a conceptual framework for scaling measured metabolic rates using data collected at patch and reach scales across multiple sites and years on Konza Prairie Biological Station. I evaluate the effectiveness of scaling with considerations for abiotic and biotic factors, including the

experimental removal of fish at both scales, and other factors that should be considered when up-scaling metabolic rates in steams. This is the first study to explicitly compare metabolic rates in streams across multiple spatial scales. In the second chapter, I analyzed patch-scale data from the previous chapter using path analyses to determine the direct and indirect effects of fish presence and multiple abiotic factors on GPP, ER, and ammonium uptake. Path analysis is an underutilized tool in ecology, and has the advantage over multiple regression approaches in that it allows more complex interactions among variables to be evaluated. Few studies include both biotic and abiotic factors simultaneously when determining drivers of biogeochemical rates.

Chapter 2 - Scaling nested measurements of biogeochemical rates across prairie stream reaches with varying biotic and abiotic characteristics.

Abstract

Understanding the variability of ecological processes across spatial scales is a central issue in ecology, because increasing scale is often associated with increasing variability and complexity. Furthermore, ecological measurements are often made at scales smaller than those that management decisions are made. We generated a conceptual framework with predictions for scaling biogeochemical rates and tested our predictions by comparing measurements of ecosystem respiration (ER) and gross primary production (GPP) from patch to reach-scales (benthic areas of 1-100 cm^2 or 10-100 m^2, respectively) over a two-year period across multiple sites (n= 7 for each rate). We evaluated interactive biotic and abiotic effects among patches by measuring biogeochemical rates in riffle and pool habitats with and without consumers, while quantifying a variety of abiotic variables (organic matter, water velocity, light availability, and substrata size) in each habitat. Consumer removal did not alter metabolic rates at the patch scale, suggesting abiotic conditions are more important drivers of these processes. Rate measurements in patches significantly overestimated reach ER and GPP after corrections for habitat heterogeneity, stream conditions (i.e. temperature and light) at the time of measurement, and abundance of alternative stream compartments (i.e. macrophyte beds and leaf packs). Our inability to scale is likely affected by different methodological approaches since reach scale measurements were *in situ*, while patch-scale measurements required removal of incubated substrata from the stream. Stream conditions were altered by drought and flood between years, and this translated into different up-scaled results, indicating that future climate scenarios should

be considered when making predictions at any scale. While spatially explicit scaling approaches (i.e. mechanistic or process based modeling) may have been more effective for up-scaling metabolic rates in this system, this study provides valuable insight to factors that should be considered when attempting to scale ecosystem processes in any system.

Introduction

Understanding the variability of ecological processes across spatial scales is a central issue in ecology (Levin 1992). The interpretation of ecosystem processes can be dependent on the scale with which they are measured, and translating measurements across scales cannot be assumed to be additive (Wu 1999, Thorp 2006). Most field measurements of stream processes are made at scales with benthic areas of 1-100 cm^2 or 10-100 m^2 (referred to here as patch and reach, respectively; Hauer and Lamberti 2006) due to logistical constraints, while decisions concerning the management of entire ecosystems are often made at the watershed scale (>10 km^2, Duffy 2009).

The disconnection between scales that we measure and manage can be problematic because with increasing scale comes increasing complexity and variability (Hewitt et al. 2007). In freshwater streams, unidirectional flow adds more complexity to the concept of scale because organic matter, nutrients, and aquatic organisms can readily move (or cycle) at the watershed scale (e.g., Schlosser 1991, Fausch et al. 2002, Banner et al. 2009). Abiotic and biotic factors often interact uniquely at different scales to affect a variety of stream ecosystem structural and functional characteristics. Furthermore, species evolve in response to abiotic selective pressures that vary from patches to entire watersheds or larger.

For this study, we focus on scaling basic biogeochemical processes (e.g. respiration and primary production) from patches to reaches, by accounting for the primary consumer feedbacks on these processes (e.g. bioturbation, grazing, and excretion by aquatic organisms; Berke 2012) and how they interact with important stream abiotic factors (substrata, water velocity, light availability, and organic matter). Both consumer presence and abiotic factors at the patch-scale will be important when attempting to predict (i.e. scale) biogeochemical rates at larger spatial areas. We created a conceptual framework of the hypothesized primary consumer effects and

abiotic drivers of multiple structural and functional components across patch, and reach scales (Figure 2.1, Obj. 1 and 2) for the Kings Creek watershed on Konza Prairie Biological Station (KPBS), and evaluate these predictions with measured data.

At the patch-scale, the total effect of interactive consumer effects and abiotic factors will likely be habitat specific (riffle or pool; Pringle et al 1988, Vanni 2010). The magnitude of abiotic effects may differ based on the habitat type and variable of interest (e.g. stream velocity, light availability), while consumer effects will be dependent on the biomass of organisms present in each habitat. Fish abundance and diversity in prairie streams tend to be higher in pools compared to riffles (Martin et al. 2013); however, the magnitude of this effect and its interaction with abiotic factors will likely determine the total effect on the stream ecosystem (Figure 2.1).

As an example of consumer effects on ecosystem structure and function across scales we consider the relationship between fish with algal biomass and gross primary production (GPP) rates, which are inherently linked and can be affected by similar biotic and abiotic drivers (Vanni et al. 2006, Bernot et al 2010). Fish can affect local algal biomass standing stocks and production rates through grazing (Murdock et al. 2011, Berke 2012). Any grazing effects would likely interact with local light availability and velocity, which are major abiotic drivers of these variables (Mulholland et al. 2001, Bernot et al. 2010), especially at patch scales. We predict that light availability will vary minimally within a given reach, since stream width varies minimally between habitats in headwater streams, while velocity and fish standing stocks (and their effects) would be dependent upon stream habitat. These predictions suggest that we should be able to scale from smaller to larger scales if we can account for habitat heterogeneity in up-scaling calculations. Fish excretion can also be important for stream ecosystems (McIntyre et al. 2008), especially in nutrient-limited prairie streams (Gray et al. 1998). However, given that the uptake

length (the distance a molecule travels before being taken up by the benthos) of nutrients is often the length of a typical reach-scale measurement (e.g. 24-58 m in Dodds et al. 2002 measured with ^{15}N at ambient concentrations; also see Bertrand and Gido 2007), effects of mineralization would likely be transmitted to downstream reaches and require larger scale consideration (e.g. multiple reaches or watersheds).

There are other important methodological factors to consider when up-scaling stream structural and functional characteristics, which are often dependent upon the method used to estimate a variable of interest at each spatial scale. For example, reach-scale estimates of algal biomass can be estimated by averaging multiple patch-scale estimates and reporting values as either a standing stock density (i.e. mg chlorophyll *a* m^{-2}) or as an areal standing stock by multiplying the density by the steam area (mg chlorophyll *a* $reach^{-1}$). Stream complexity and habitat heterogeneity will likely dictate where and how many samples are necessary to capture an accurate reach-scale measurement. In contrast, reach-scale estimates of GPP can be estimated using whole stream methods, with a calculation approach that is tailored to measuring primary production across a large streambed area. Stream heterogeneity is less of an issue for whole stream methods; however, replication for these methods is often low with limited ways to calculate measurement error. Methodological details must be considered alongside the major biotic effects and abiotic stream conditions when attempting to scale variables.

Herein, we create a scaling framework that accounts for scale-specific factors to evaluate the relationship between metabolism rates and their primary biotic and abiotic drivers measured at the patch and reach-scales (Figure 2.1). Assessing measurements across scales while accounting for the important biogeochemical drivers is an important first step towards generating accurate rates at larger scales. We tested predictions of differences in patch-scale abiotic stream

characteristics by comparing measurements from riffle and pool habitats (Figure 2.1, Obj. 1). Predictions of consumer effects on stream structure and function were tested by manipulating fish presence at patch and reach-scales (Figure 2.1, Obj. 2). Metabolism measurements from both patch and reach scales were directly compared to evaluate the effectiveness of scaling using our predictions from Obj. 1 and 2 and other important factors (Figure 2.1, Obj. 3). *Our overarching hypothesis was that experimental measurements of metabolic rates at patch-scales can be scaled to reach measurements if we can account for habitat distribution (pools and riffles), fish biomass within the reach, available substrata (FBOM, leaf pack, etc.), and environmental conditions (e.g., temperature, light) at the time of measurement.*

Methods

Study Areas and Site Selection

This research was conducted in the Kings Creek watershed of the KPBS, which is located within the Flint Hills ecoregion, and is characterized by rolling hills, with cherty limestone and shale outcrops. The Flint Hills are the largest extent of intact native tallgrass prairie in the Great Plains (Omernik 1987). The KPBS is a 35 km^2 nature reserve and Long-Term Ecological Research (LTER) site owned by The Nature Conservancy and managed by the Kansas State University Division of Biology. Watershed-level treatments include prescribed burning (with burn frequencies of 1, 2, 4, and 20 years in sub-watersheds), and grazing by the American bison (*Bison bison*; 1/3 of total area openly grazed by bison). Kings Creek is an intermittent stream and is subject to frequent and severe floods and drought (Dodds et al. 2004). Kings Creek has been extensively studied for both natural and experimental multi-scale measurements of nitrogen cycling (reach-scale: Dodds et al. 2002, O'Brien et al. 2007; patch-scale: Kemp and Dodds 2002, O'Brien and Dodds 2008) and metabolism rates (Reach-scale: Riley and Dodds 2012, Riley and

Dodds 2013; patch-scale: Murdock et al. 2010, Wilson and Dodds 2009), though not at both scales simultaneously.

Experimental Design

We chose 3 sites to measure reach and patch metabolic rates in late spring of 2013 and 2014 (n=7 total reaches, Figure 2.2). Two of the sites were sampled in both 2013 and 2014 years (E1 and E3), while a third site was sampled only in 2014 because the stream was dry at this site in 2013 (E2). Sites were selected based on water and flow availability and the locations of previously collected data. A list of characteristics for each reach and year can be found in Table 2.1. Here, we are using the term 'patch' to represent measurements taken at our smallest scale (e.g. benthic area of ~300 cm^2), and 'reach' to represent measurements taken across a whole reach (e.g. benthic area ~ 100 m^2). The length of each reach (33-60m) was determined based on a 20-30 minute travel time (i.e. the time it takes a parcel of water to travel a given distance), which is ideal for accurately measuring reach-scale metabolism rates in a constrained reach in this system. At each site, patches were distributed within a reach based on the total percent of each habitat type. For example, if the reach was 40% riffle then 4 of the 10 patch measurements would be placed in different riffles spread throughout the reach. Each patch consisted of five plastic containers (i.e. strawberry baskets; 11.4 cm x 9.5cm x 6.6 cm) filled with substrata representative of the stream. The containers had an effective mesh size of 1 cm and were capable of holding most pebble sizes. Final measurements were taken at both scales following a 30-day incubation period in the steam. The location of the patches relative to the reach-scale measurements varied between years.

2013 measurements

In 2013, we implemented an experimental manipulation of fish at both patch and reach scales. The design of this experiment included three adjacent but separated reaches at two sites (E1 and E3; Figure 2.2). Each site (in longitudinal order from upstream to downstream) consisted of an 'ambient' reach where ambient fish biomass were retained, a 'patch' reach where fish and crayfish (herein referred to as 'consumers') presence was manipulated at the patch-scale using constructed exclosures, and a 'removal' reach where consumer standing stocks were reduced (Figure 2.3 A). Reach-scale measurements were conducted in the ambient and removal reaches, while patch-scale rates (with and without consumers) were measured in the patch reach. At the top and bottom of the ambient and removal reaches, wire mesh (height=1.1 m, mesh interval= 1cm) was placed across the stream and buried in the streambed. The fence was also zip tied to iron bars driven vertically into the stream sediment placed in intervals on the downstream side of the fence. We enclosed the ambient reach with fencing to ensure that consumer biomass did not change across the 30-day incubation period and mimic any enclosure effects in the removal reach. Eight sets of two substrata containers were placed in the ambient and removal reaches across multiple riffle-pool sequences, and was used to quantify standing stocks of fine benthic organic matter (FBOM) and algal biomass for comparison with patch exclosure measurements.

We used a combination of electro-shocking and seining to remove consumer from each reach across multiple (no less than three), equally timed passes. Each consumer was identified to species and its length measured for calculation of biomass from previously established length-weight regressions (Keith Gido, personal communication; Appendix B). We continued to remove consumers from the removal reach until a significant portion of the biomass was removed, releasing consumer downstream after processing. Consumers removed from the ambient reach were retained and returned to the reach after a significant depletion. All reaches were

periodically checked for consumer presence during the incubation period. If present, they were removed with one or two passes of electroshocking. Any extra passes completed in the removal reach were simulated in the ambient reach. After final measurements at the end of the experiment, we evaluated the effectiveness of our treatments by repeating multiple passes of electroshocking for final estimates of consumer populations and total biomass. The total number of individuals for each species was determined through maximum-likelihood multi-pass population estimates (Hayes et al. 2007) for all reaches. Population estimations were then used to calculate total biomass within each reach at the beginning and end of the experiment using length-weight regressions. The average length of each species within a reach was used to calculate the average biomass of an individual of that species and then multiplied by the population estimate for total species biomass. Total reach biomass was divided by the reach area to attain consumer biomass.

Substrata containers for patch-scale measurements were put inside and outside of eight constructed exclosure devices (n=16 total patch measurements per site) spread throughout the patch reach. Each exclosure consisted of a square wooden frame (50 cm x 50 cm) wrapped with hardware mesh (same as reach fences). The exclosures were fashioned so that half the frame (e.g. a triangle) was completely enclosed with mesh (consumers not present) while the other half was only enclosed on the upstream edge so that any alterations to flow and light would be similar across treatments (consumers present, see Figure 2.3 B). Five substrata filled baskets were placed in each side of the exclosure and gravel was filled in around them to mimic natural streambed conditions. The exclosures were buried in the stream so that the top of substrata containers would be flush with the streambed.

Low discharge and lack of a high enough flow to move fine sediments and leaves in the 2 years before the start of this experiment resulted in abnormal stream conditions at E3 in 2013 that had not been observed in the last 20 years (Walter Dodds, personal communication). Most notably, leaf packs and macrophyte beds were exceptionally abundant compared to a typical year. Therefore, we quantified patch-scale metabolism measurements using similar methods as the substrata containers from both of these compartments because of their potential contribution to reach-scale metabolism rates.

2014 measurements

The experimental manipulation of consumer presence could not be duplicated in 2014 because a flood destroyed the equipment necessary for consumer exclusion. We only used the equivalent of the 2013 'ambient' reach at three sites in 2014. A third site (E2) was added because of increased flow after the flood, while the other two sites (E1 and E3) where the same across years. The three reaches measured in 2014 were not enclosed in fencing and patch containers (n=10) were placed directly into the reach across multiple riffle-pool sequences.

Ecosystem Rates

Patch-scale measurements for gross primary production (GPP) and ecosystem respiration (ER) were measured consecutively at each experimental site using sealed acrylic chambers (~15 L total volume) with an internal propeller driven circulation system (Rüegg et al. *In Press*). Three of the five baskets filled with incubated substrata were carefully removed from each exclosure or patch transect and transported in a container to a chamber. Each chamber was filled with stream water of known volume, sealed, and a ProODO meter (Yellow Springs Instruments, Yellow Springs, OH) attached. Dissolved oxygen concentrations were logged for approximately 30 minutes. The first half of that time the chamber was covered with a light impenetrable fabric

to estimate ER. During the last 15 minutes the chamber was open to ambient light to measure net ecosystem production (NEP). Light and temperature were monitored throughout the incubation period to quantify changes during the measurement period for later corrections in rate calculations.

Replicate (n=4) patch-scale metabolism rates were measured from macrophytes and leaf packs at E3 in 2013 using similar methods, and were only applied to scaling measurements to E3 ambient and removal reaches as they had high abundances that year. Macrophytes and leaf packs were transferred directly to the chamber from the stream. Leaf packs were weighed down with dry rocks from the stream bank if necessary. Point estimates of each compartment (silt/gravel, macrophytes, and leaf packs) were conducted at 10 points across 10 transects to calculate the percent of each compartment within the reach for later weighting of rates.

Reach-scale metabolism was measured in 2013 and 2014 using either a single or two station methods with modeled reaeration values (see below for calculation methods). Dissolved oxygen was measured using ProODO dissolved oxygen meters attached to rebar at mid-depth in an area of flow constriction and in the thalweg of the stream at the top and bottom of the reach. Prior to installation and after removal, probes were calibrated at 100% oxygen saturation and then allowed to log for 30 minutes in a bucket or in a similar location in the stream to compare meter drift. Light meters (Odyssey PAR logging meters, Dataflow Systems Pty Limited, Chirstchurch, New Zealand) were attached and leveled at the top of the rebar above each ProODO meter. Both meters were set to log simultaneously in 10-minute intervals. Reach-scale velocity estimates were calculated using travel time (measured from NaBr or Rhodamine releases) and reach length.

Ancillary Data

Before the beginning of the experiment in 2013 and 2014 canopy cover, velocity (2013 only), substrata size, and habitat heterogeneity were measured at 7-10 transects (approximately 5m apart) in each ambient and removal reach. Canopy cover was measured using a spherical densitometer in the middle of the stream at each transect. Patch-scale velocity was measured across 10 transects within three reaches in 2013 using an ADV Doppler meter. Substrata size was measured using a gravelometer, with a minimum of 20 particles measured evenly throughout each transect. Habitat heterogeneity was determined through qualitative assessment of stream conditions (i.e. velocity, depth) at each transect.

Algal biomass (as density of chlorophyll a) and FBOM standing stocks were measured once (same day as patch biogeochemical rate measurements) from each reach and patch transect each year from one of the remaining incubated baskets. Chlorophyll *a* concentrations were calculated from 3-5 rocks taken from the top of the basket. The rocks were stored in a whirl pack and frozen until thawed for hot ethanol extraction (78°C for 5 minutes, followed by 12 h at 4°C; Sartory and Grobbelaar 1984) and analyzed fluorometrically (Welschmeyer 1995) within 30 days of collection. Standing stocks were scaled to total rock surface area as measured through projected image analyses. The remaining substrata from the same basket was then submersed in a bucket with 5 L of stream water. The substrata was agitated to suspend benthic organic matter and a 500 mL subsample was taken from the slurry and kept on ice or refrigerated until analysis within 48 hours of collection. Standing stocks of FBOM were determined by filtering a known volume of the subsample through an ashed Whatman GF/F 0.7 μm filter and dried at 40º C (dry mass; >48 hours) and 460º C (ash free dry mass; >3 hours). The mass of FBOM was scaled to total basket surface area (0.01 m^2).

Data Analyses

Patch Rates

Preliminary patch biogeochemical rates were calculated using equation 1 (O'Brien and Dodds 2008):

$$U = M \times V \times A^{-1} \quad \text{eqn. 1}$$

Where *M* is the change in oxygen concentration ($[O_2]$) over a known amount of time, *V* is the volume of the chamber after substrata addition, and *A* is the area of the substrata (always 300 cm^2). A variety of standardizations and corrections were applied to these rates before comparison to reach rates. Individual GPP and ER patch rates were first standardized to similar rates at 20ºC using correction equations from Parkhill and Gulliver (1999). Furthermore, GPP measurements were corrected to 300 PAR (Photosynthetically Available Radiation; µmol photons m^{-2} s^{-1}) using data from a photosynthesis-irradiance curve (PI curve, Appendix A) and correction coefficient 1 (equation 2). GPP rates used in correction coefficient 1 were calculated from the equation modeled by the PI curve based on 300 PAR and the PAR at the time of measurement in the chamber (i.e. they are not measured GPP rates).

$$\text{Correction coefficient 1} = \frac{calculated\ GPP\ at\ 300\ PAR}{calculated\ GPP\ at\ measured\ PAR} \quad \text{eqn. 2}$$

Correction coefficient 1 was multiplied by GPP rates that *were* measured in the chambers to correct all GPP rates for varying light at the time of measurement. At this point, ER values were in units of mg O_2 m^{-2} min^{-1} at 20° C and GPP in units of mg O_2 m^{-2} min^{-1} at 20° C and 300 PAR (Photosynthetically Active Radiation). Patch rates were weighted by the percentage of habitat (i.e. percent of pools and riffles) of the reach using equation 3:

Habitat-weighted rate =

$$(average\ riffle\ rate\ \times\ \%\ benthic\ riffle\ area) + (average\ pool\ rate\ \times\ \%\ benthic\ pool\ area)$$ eqn. 3

Habitat-weighted rates were corrected for stream temperature (ER and GPP) and light (GPP) conditions using data from reach-scale metabolism calculations (measured at 10 minute intervals) over a 24-hour period, for a total of 144 time points (6 x 24=144). The habitat-weighted rate from equation 3 was corrected for reach temperature and/or light at each time point. Patch ER and GPP rates were first corrected for stream temperature using the same temperature conversion equations as above. GPP was then scaled to reach-scale light conditions using correction coefficient 2 (equation 4). This correction coefficient scaled the temperature-corrected and habitat-weighted GPP patch rates to light conditions found in the stream during reach-scale measurements at each time point.

Correction coefficient 2= $\frac{GPP\ at\ reach\ light}{calculated\ GPP\ at\ 300\ PAR}$ eqn. 4

All of the rates (n=144) were then summed across the 24-hour period. Daily patch-scale metabolism rates from E3 in 2013 from each compartment (silt/gravel from containers, macrophytes, and leaf packs) were weighted by the percent of that compartment inside the reach with equation 5.

Compartment-weighted rate= eqn. 5

$$[(scaled\ silt/gravel\ rate\ \times\ \%\ benthic\ silt/gravel\ area) + (scaled\ leafpack\ rate\ \times\ \%\ benthic\ leaf\ pack\ area) + (scaled\ macrophyte\ bed\ rate\ \times \%benthic\ macrophyte\ bed\ area)]$$

Reach Rates

We estimated stream metabolism using both single and two station methods (Marzolf et al. 1994, Young and Huryn 1998) by modeling the change in $[O_2]$ at each10 minute data point with temperature and light corrections according to the studies of Parkhill and Gulliver (1999, temperature) for respiration and GPP and Jassby and Platt (1976, GPP light saturation). Reaeration coefficients were temperature corrected according to Elmore & West (1961) and Bott (2006). The equation describing the dynamics of $[O_2]$ is

$$\frac{d[O_2]}{dt} = k_{20} \times 1.024^{T-20}([O_2]_{sat} - [O_2]) - R_{20} \times 1.045^{T-20} + P_{max} tanh(\frac{\alpha I}{P_{max}}) \times 1.036^{T-20} \quad \text{eqn. 6}$$

Where k_{20} is the standardized reaeration coefficient (min^{-1}) at 20°C, T is temperature in the stream, $[O_2]_{sat}$ is dissolved oxygen concentration in equilibrium with the atmosphere, R_{20} is the standardized respiration rate at 20°C, P_{max} is the photosynthesis rate at light saturation, I is light intensity, and α is the slope of photosynthesis-light relationship at low light intensity. We employed a Bayesian approach for parameter estimation. For the one station method, we formed the likelihood function by assuming a normally distributed observation error between modeled and measured $[O_2]$. To obtain the estimated $[O_2]$, we interpolated light and temperature linearly between measurement times and solved equation 1 using differential equation solver "*lsoda*" in R (Version 3.1.2; R Project for Statistical Computing, Vienna, Austria) package "*deSolve*" (Soetaert et al. 2010). For the two-station method, we formed the likelihood function by assuming a normally distributed observation error between modeled and measured changes of $[O_2]$ from upstream to downstream. When calculating the modeled changes in $[O_2]$, we did not directly solve equation 1 because it involves estimating a specific initial condition for each measurement of changes in $[O_2]$ from upstream to downstream, which resulted in an excessive amount of parameters to model. Instead, we approximated light, temperature, $[O_2]$, and $[O_2]_{sat}$

using a linear interpolation between measurements at the top and bottom of the reach lagged by travel time and integrated the right hand side of equation 1 to obtain the modeled changes in $[O_2]$. We used uniform distribution as prior for all parameters and implemented the adaptive random walk Metropolis-Hasting algorithm (Haairo et al. 2001) to sample the posterior distribution. We performed Geweke diagnostic (Geweke 1992) and visual inspection to ensure convergence of the Markov Chain. The mean of the posterior distribution served as the point estimates of each parameter. The highest posterior density interval was used for the interval estimates of each estimated parameter.

We obtained the daily O_2 production or consumption due to GPP or ER by integrating the modeled instantaneous photosynthesis rate and respiration rate over the time of a day. For two-station method, we used the average temperature and light between top and bottom of the reach when calculating daily GPP and ER. The interval estimates of daily respiration can be obtained by using the lower and upper bound of R_{20} interval estimates in integration. For interval estimates of GPP, we use the lower and upper bound of α in integration when the site is not light saturated. If a site was light saturated, we calculated the interval estimates of GPP by Monte Carlo integration. This was done by sampling P_{max} and α repeatedly with replacement from the posterior distribution and calculates the GPP for each pair of P_{max} and α.

Statistical Analyses

Analysis of variance (ANOVA) was used to test predicted differences (Figure 2.1) of patch-scale abiotic physical characteristics (canopy cover, velocity, CBOM, and substrata size) between habitat types from reach transects in both ambient and removal reaches from E1 and E3 2013 (Figure 2.1, Obj. 1). Reach-scale standing stocks were compared with patch-scale measurements from 2013 using a Student's t-test since these data were collected using similar

methods in different reaches (patch vs. ambient or removal reach) and are known to be important local drivers of biogeochemical rates.

Two-way ANOVAs (one for each rate and both standing stocks, n=4) were used to test predicted consumer effects from patch-scale consumer manipulations in 2013 and the possible interaction with stream habitat type (Figure 2.1, Obj. 2). Non-normal response variables, as indicated through the Shapiro-Wilk Normality Test were natural log transformed.

We used linear regression to predict reach scale biogeochemical rates from patches in 2013 and 2014 (Figure 2.1, Obj. 3). We assume that reach-scale measurements are the 'accurate' measurement since the rates are integrating entire reaches and our overall goal is to predict rates at larger spatial areas. Regressions were forced through the origin, with the assumption that rates at both scales would be zero during certain conditions (e.g. a desiccated stream-bed). The 95% confidence interval (CI) of the slope from each regression was compared to a slope of one (i.e. the slope where values between independent and dependent variables are the same). If the CI of the slope did not overlapped with one, the measurements at each scale were considered to be statistically different. All analyses were conducted in R (version 2.15.1).

Results

Environmental and structural conditions

Five of the seven reaches in this study were dominated (>50%) by riffles (Figure 2.4). The two reaches with higher proportions of pools both occurred at E3. Average reach canopy cover varied between 47% and 81% among sites, but there was not a statistical difference between riffle and pool habitats across (Figure 2.5 A; $F=0.19$, $p=0.67$). The highest average canopy cover occurred at E2, which also had the thickest riparian vegetation and narrowest stream width. There was no evident watershed scale gradient of canopy cover. Patch-scale

velocity measurements ranged from 0- 0.31 m s^{-1} across the three reaches measured in 2013 (E1 ambient reach wasn't measured). Velocity was significantly higher in riffles (Figure 2.5 B; $F=10.31$, $p<0.01$), with only one pool with a detectable velocity greater than zero. Stream discharge was much lower in 2013 than 2014 across all sites at the time of metabolism measurements, ranging from 0.5-0.7 L s^{-1} in 2013 and 4.7-10.2 L s^{-1} in 2014 (Table 2.1). Course benthic organic matter (CBOM) was significantly higher in pools compared to riffles (Figure 2.5 C; $F=17.2$, $p<0.001$). Median substrata size was not significantly different between habitat types (Figure 2.5 D; $F=1.59$, $p=0.21$).

Reach-scale consumer removal in 2013

Total consumer biomass before fence installation was 10.6 g m^{-2} for the ambient reach and 5.2 g m^{-2} for the removal reach at E3 (Table 2.2). As many as eight species were identified from the E3 site, with the Central Stoneroller (*Campostoma anomalum*) and the Southern Redbelly Dace (*Phoxinus erythrogaster*), both benthic grazing minnows, contributing 82% of the total biomass in the ambient reach and 77% in the removal reach. We were able to reduce consumer biomass in the removal reach by 67% of pre-treatment levels to a biomass of 1.7 g m^{-2} during the length of the experiment. *Campostoma anomalum and P. erythrogaster* biomass consisted of 67% of the total biomass at the end of the experiment in the removal reach. Total biomass in the ambient reach increased 4% during the incubation.

Biomass in E1 was much lower than E3, with total biomass between 0.14-0.26 g m^{-2} across both reaches (Table 2.2). As many as four species were identified at the E1 site, with *C. anomalum* and *P. erythrogaster* consisting of 67% of the total biomass in the ambient reach and *P. erythrogaster* alone (*C. anomalum* was not observed in this reach) consisting of 69% of the total biomass in the removal reach before the experiment. Both reaches at E1 gained biomass

during the length of the experiment, with an increase of 0.21 g m^{-2} and 0.14 g m^{-2} in the ambient and removal reaches, respectively (Table 2.2).

Standing stocks

There was no evident effect of consumer removal or habitat type on FBOM at E1 (Figure 2.6 A, Table 2.3). There was a marginal increase of FBOM standing stocks after consumer removal at E3 in 2013 ($p=0.11$; Table 2.3, Figure 2.6 B), and significantly higher FBOM standing stocks in pools compared to riffles ($p=<0.05$, Table 2.3, Figure 2.6 B). Algal biomass was not different between habitats, and was not altered by consumer removal at either site (Figure 2.6 C-D, Table 2.3). In 2014, E1 had the lowest and least variable FBOM standing stocks, while E2 had the highest average and the most variable standing stocks (Figure 2.7 A). Algal biomass was equally higher at E1 and E3 compared to E2 (Figure 2.7 B).

Standing stocks of FBOM measured across both scales were statistically similar ($p>0.05$) in both removal and ambient measurements at E3 in 2013 (Figure 2.8 A). FBOM values in the ambient reach at E1 overestimated the FBOM values from the open side of the exclosure in the patch reach. FBOM standing stocks were observably higher at E3 compared to E1 (non-statistical observations are presented here for differences between sites due to lack of replication). Algal biomass was statistically similar across scales with both ambient and removal measurements at E3 (Figure 2.8 B). At E1, patch-scale algal biomass was significantly higher in the reach compared to the closed side of the exclosure in the patch reach. Algal biomass varied minimally across sites (Figure 2.8 B). Reach measurements of CBOM were statistically higher ($p<0.05$) than patch-scale exclosures for all reaches regardless of site and consumer manipulation (Figure 2.8 C).

Metabolism rates

There were no statistical differences for patch-scale metabolism measurements between habitats or consumer removal treatments in 2013 at either site (Figure 2.9 A-D, Table 2.3). Respiration rates were notably higher at E3 compared to E1, while GPP rates were similar across sites. Daily GPP rates ranged from 1.5-8.3 g O_2 m^{-2} day^{-1} in 2013 and 3.5-13.4 g O_2 m^{-2} day^{-1} in 2014. Daily ER rates ranged from 0.39-3.5 g O_2 m^{-2} day^{-1} in 2013 and 1.9-6.1 g O_2 m^{-2} day^{-1} in 2014. Macrophytes contributed 57% of the estimated daily GPP rates in the E3 2013 removal reach, and 34% in the ambient reach (Figure 2.10 A). Estimated daily ER rates from silt/gravel containers were similar between consumer manipulations. The macrophyte compartment dominated the rest of the estimated ER, with a minimal contribution from leaf packs (Figure 2.10 B). Macrophytes and leaf packs were almost completely absent in 2014 measurements after the flood.

Reach-scale ER was highly variable between E1 reaches in 2013, with lower rates in the ambient reach (0.09 g m^{-2} d^{-1}) compared to the removal reach (3.7 g m^{-2} d^{-1}). The opposite occurred in E3 reaches, where rates were ~1.2 g m^{-2} d^{-1} higher in the ambient reach relative to the treatment reach (Table 2.4, Figure 2.11). Between years, ER was similar (within 0.5 g m^{-2} d^{-1}) in reaches with ambient consumer biomass at both sites. Reach-scale GPP rates were much less variable across sites, especially in 2013. Treatment and removal reaches exhibited similar rates in E3, while the removal reach rates were about two times higher than the ambient reach in E1. Rates in 2014 where variable across sites, with higher rates at the lowest site (E3) in the network and lowest rates at the highest site in the network (E1; Table 2.4, Figure 2.11).

Patch-scale metabolism rates overestimated reach-scale rates in most reaches. However, there was a significant relationship between measured scales ($p<0.05$ for both rates), and they were highly correlated ($R^2 > 0.80$ for both rates; Figure 2.11). Regression slopes were

significantly less than one for both rates, with slopes of 0.30 for ER (95% CI=0.09-0.51) and 0.24 for GPP (95% CI=0.09-0.39). Reach-scale rates overestimated patch-scale rates in three of the 14 measurements (including both rates), two of which occurred with consumer removal measurements (Figure 2.11). For both GPP and ER, patch and reach-scale rates were more similar at the lowest measured rates, while at higher rates the patch-scale overestimation was more notable.

Discussion

Effectiveness of consumer removal

Patch-scale consumer removal was effective in removing all consumers less than 1 cm in body size. Experiments removing or enclosing stream organisms at patch and habitat scales are common and can be done using a variety of methods including: plastic screening (Power 1992), wire mesh (Power et al. 1985, Bertrand and Gido 2007, Bertrand and Gido 2009, Murdock et al. 2010), electric fields (Pringle and Hamazaki 1997, Effenberger et al. 2011), and tethering individual consumers to the benthos (Power and Matthews 1983). Studies conducted at scales smaller than whole habitats have the advantage that control and treatment manipulations can be replicated within the same stream transect with similar abiotic and biotic conditions; however, the smaller benthic area might limit the applicability of results.

While studies at the patch-scale are ample, few mention the effect of cage enclosures or exclosures on experimental results (see Power 1992). We anticipated quantifying possible cage effects by comparing measurements from within each side of the exclosure to baskets placed immediately upstream of the exclosure; however, exclosures were lost during a flood. Even so, we attempted to mitigate any cage effect by enclosing the upstream end of the ambient side of

the exclosure with wire mesh, so that any effects on flow velocity or shading would be similar across experimental units.

Studies manipulating stream organisms at the reach-scale are less common than experimental patch-scale manipulations. Taylor et al. (2006) completely removed a migratory detritivorous fish, *Prochilodums mariae*, from half of a tropical stream by inserting a wire mesh fence in the middle (and parallel to) the stream. They detected significant decreases in the downstream transport of organic carbon, and increased primary production and respiration. We effectively reduced consumer biomass standing stocks by 67% in the E3 removal reach in 2013 from 5.2 to 1.7 g m^{-2} before and after the treatment, respectively. Replication of patch and reach-scale consumer removal for this experiment was reduced from six sites to one because of drought conditions in 2013 (2 instead of 3 sites), abnormally low consumer biomass at E1 in 2013, and loss of equipment in a flood for the second year of sampling. Therefore, the scope of interpretation of consumer removal results is limited. Despite this, our reduction of consumer at this site was simple and inexpensive. Larger animals with longer generation times are usually more sensitive to anthropogenic disturbance so understanding the cascading effects of removing those organisms may require similar experiments in other biomes and across seasons.

Scaling Ecosystem Rates

Patch and reach-scale rates of ER and GPP measured in this study were typically in the lower range of measurements from recent studies conducted in headwater streams of KPBS (Riley and Dodds 2012, Riley and Dodds 2013). Uncorrected patch metabolism rates in this study matched those from Murdock et al. (2010) and Bertrand et al. (2009, GPP only) from KPBS despite these studies using different measurement chambers (Dodds and Brock 1998) and

measurements from a mixture of natural streams and stream mesocosms with varying experimental treatments.

Patch-scale rates overestimated reach-scale rates for both GPP and ER using habitat-weighted additive scaling, after accounting for corrections of temperature and light conditions at the time of measurement, and metabolism rates from other substrata compartments (Figure 2.11). Below we evaluate predictions from our scaling framework and their implications for scaling metabolic rates in streams.

Objective 1—Observed abiotic characteristics between stream habitats

We observed the natural variation in 4 abiotic stream characteristics across riffle and pool habitats in reaches adjacent to experimental consumer manipulations. These variables were chosen based on their likelihood for affecting biogeochemical fluxes. There was not a significant difference in canopy cover between stream habitats. Differences in canopy cover within a given stream reach might be expected when tree fall creates gaps in the canopy (Pringle et al. 1988), or with drastic differences between stream width between riffle and pool habitats. The widths of headwater streams in Kings Creek vary minimally between stream habitats. Lower in the watershed (i.e. at E3), stream width between habitats can vary substantially; however, these reaches are also heavily incised, adding shading where the canopy cover may not.

Stream velocity was significantly lower in pools compared to riffles, with only one of the 10 pools having a measurable velocity in 2013. Discharge at E3 never exceeded 1.5 L s^{-1} during the incubation period, and was 0.5 L s^{-1} when measurements were taken at the end of the experiment, with similar decreases in discharge found at E1 across the incubation period.

Course benthic organic matter (CBOM) was significantly higher ($p<0.05$) in pools compared to riffles, but there was not a significant difference in median substrata size between

habitats. Both substrata size and CBOM can be susceptible to the magnitude of stream flow. Flow variability as a result of varying climatic conditions (i.e. drought and flood events) has been attributed to the accumulation of organic matter during low flow and losses during major floods in intermittent Mediterranean streams (Acuña et al. 2004). Large substrata require much higher flow to be displaced. On KPBS, flood events that reach approximately 80 L s^{-1} are capable of displacing small rocks and fine sediments, while events greater than 500 L s^{-1} can displace large rocks (Dodds et al.1996). The lack of a flood or spate in the 2 years prior to this study and low stream velocity likely resulted in higher abundances of CBOM settling in pools and fine sediments settling in all areas of the stream. The presence of more than usual fine sediments in riffles likely resulted in similar median substrata size between stream habitats.

Overall, the differences of some abiotic variables in these reaches validate the assumption that patch-scale rates should be weighted by the variation of abiotic characteristics. We used stream habitat, which was determined qualitatively based on observed stream conditions, as a proxy to represent the variation of abiotic characteristics; however, we did not detect a significant difference in metabolic rates between habitats. There are alternative methods that could be used to quantitatively account for the variation of drivers of biogeochemical rates. For example, Sakamaki and Richardson (2013) found thresholds associated with stream width and fine particulate organic matter (FPOM) standing stocks, which is a proxy of biogeochemical rates in their system. Thresholds of any abiotic stream characteristic could be used to weight measured biogeochemical rates at a specific spatial scale, and in some systems may be a more accurate method of accounting for variation than stream habitat.

Objective 2—Effects of consumer removal on stream structure and function

There was no effect of patch-scale consumer removal on standing stocks or metabolic rates at E1in 2013, likely because consumer biomass were generally low (Table 2.2). Drought conditions disconnected this reach with permanent headwater streams and concentrated consumers in limited habitats resulting in higher than average consumer biomass standing stocks (Keith Gido, personal communication). The following evaluates our predictions for consumer effects on stream structure and function at E3 in 2013 were consumer standing stocks were substantially higher.

We did not detect a significant effect of consumers on FBOM standing stocks; however, there is potential for an interactive effect between consumers and stream habitat. The mean FBOM density in pools without consumers was more than 100 g AFDM m^{-2} and was higher than measurements without consumers in riffles and both habitats with ambient consumer biomass. This difference is likely responsible for a near marginal effect of consumers on FBOM standing stocks (p=0.12). This suggests the possibility of consumers affecting available FBOM standing stocks in pools, even when stream velocity is low.

Despite significant differences in velocity between stream habitats, there was no detectable effect of habitat or consumer removal on algal biomass or GPP rates. Murdock et al. (2010) found that fish effects on GPP and algal biomass typically weaken 2 weeks after a scouring spate, likely due to the accumulation rate of algal biomass becoming greater than the fish-grazing rate over time. Our substrata baskets were filled with dried sediments from an adjacent stream bank. The succession of algae from of our substrata likely followed a similar trajectory as substrata recently scoured from a flood. Stream velocity can be positively correlated with primary production rates (Dodds and Biggs 2002), but stream velocity in the riffles of this study may not have been high enough to have a substantial effect. Similarly, *in situ*

measurements of primary production with increasing velocity in Kings Creek have failed to detect a significant relationship (Dodds et al. 1996), although other studies have noted such an effect (Horner et al. 1990, Larned et al. 2004).

We predicted that multiple abiotic factors would interact to affect ER between stream habitats, and that these factors would be more important than consumer effects. There was no significant effect of consumers on ER rates. Consumer effects on ER are likely to be mediated through consumer effects on FBOM as a result of bioturbation rather than direct effects (Trentman Thesis Chapter 3). Murdock et al. (2010) found marginal effects of consumers in Kings Creek, but these were highly variable over time during recovery from a flood event.

While we did not detect changes in metabolic rates after removing consumers from this reach, there are other aquatic animals that may also alter stream structure and function. Crayfish biomass was too low to accurately estimate reach biomass; however, crayfish can act as ecosystem engineers (Moore 2006), by hastening the breakdown of organic matter (e.g. leaf packs in Creed and Reed 2004; Evans-White et al. 2003; Whiting et al. 2011), inducing sand and gravel erosion (Statzner et al. 2000, Statzer et al. 2003), and sediment reduction (Helms and Creed 2005). Magoulick (2014) did not detect a significant effect of crayfish on metabolic rates in stream mesocosms; however, DiStefano et al. (2009) found that crayfish in intermittent streams tend to use the hyporheic zone during seasonal drying. This suggests that crayfish could play an important role on stream biogeochemistry during drought conditions by altering hyporheic nutrient concentrations through excretion, and oxygen concentrations through bioturbation. Studies investigating the interactive effects of the entire biotic community are warranted across multiple stream conditions in order to determine the implications of possible biotic effects on up-scaling metabolic rates.

Objective 3—Other factors for scaling metabolic rates

Methodological constraints

Some patch-scale overestimation can likely be attributed to differences in methods for biogeochemical rate measurements. Reach-scale metabolism measurements are entirely *in situ*, while patch-scale measurements require perturbations of the stream benthos at the end of the experiment. The removal of substrata containers from the stream for chamber measurements likely altered chemical and physical characteristics of the sample (McIntire 1966). While we attempted to preserve the patch samples, this was not always attainable.

The perturbation of sediments was likely a major factor in overestimation rates at E3 in 2013 and E2 in 2014. Three of the four sites with abnormally high patch-scale measurements were from E3, and the fourth was from E2. These sites also had some of the highest and most variable FBOM rates, with pool habitats at each site being compacted with FBOM and leaf packs. Substrata containers at both sites were immediately buried by a few centimeters of fine sediments after the beginning of the incubation. Removal of these samples altered the quantity and distribution of overlying sediments, which in turn likely altered biogeochemical conditions. For example, there is some quantitative evidence of sediment anoxia as when they were removed the odor of sulfide was detected (M Trentman personal observation). Previous studies on KPBS have detected localized zones of anoxia in Kings Creek reaches, which were associated with warm months (Kemp and Dodds 2001). Removal of sediments that were initially anoxic would significantly alter the available oxygen, and in turn affect biogeochemical rates.

We used consistent velocity for all chamber measurements (i.e. regardless of riffle or pool habitats), thus creating different physical conditions for samples from pool habitats from these sites, given that velocity in most pools was near zero. Higher velocities in the chambers may result in higher biogeochemical rates due to increased transport of overlying water across

the diffusion boundary layer (Dodds and Biggs 2002). For sites with high FBOM densities, the overlying water column may have been enriched by the suspension of nutrient rich sediments, further exacerbating effects from higher velocity.

The importance of stream structural dynamics

Algal biomass, and standing stocks of FBOM and CBOM can be primary drivers of metabolic rates (Mulholland et al. 2001, Bernot et al. 2010, M Trentman thesis chapter 3), but may vary more from reach to reach compared to the other abiotic characteristics noted above. Since measurements in 2013 were done in different reaches (i.e. patch-scale measurements were taken from a reach in-between the ambient and removal reach), it is important to compare the composition of these drivers in each reach. Significant differences of these variables between reaches may affect the ability to scale metabolic rates. Standing stock estimates of FBOM and algal biomass were similar between patch and reach-scales across most sites in 2013. FBOM standing stocks were higher in the E1 ambient reach and algal biomass was higher in the E1 removal reach compared to their respective side of patch-scale exclosures (Figure 2.8 A-B). Overall, six of the eight reaches had statistically similar standing stock densities between patch and reaches. CBOM densities were significantly higher in the reach-scale measurements than the patches for all measurements. These differences are likely attributed to the wire mesh preventing CBOM from settling within the patch exclosures. A general similarity between standing stock densities across measured scales suggest that benthic conditions were similar during the incubation period. It's likely that observed differences between measured rates across scales are not attributable to inherent differences in stream structural conditions based on 2013 measurements at sites where standing stocks were similar between reaches.

The comparability of rates across scales was typically worse when stream structure was highly variable or different between reaches (2013 only). For example, the removal reach at E1 in 2013 had significantly higher algal biomass than the patch-scale exclosure counter-part, while FBOM densities were generally low at both scales. The removal reach at E1 was the only reach where reach-scale rates overestimated patch-scale rates for both ER and GPP. It is possible that the different structural components at each scale may have attributed to this reach following a different trend than most of the other reaches. In contrast, the E3 2013 ambient measurements were closest to the 1:1 line than any other site for both ER and GPP rates (Figure 2.11), and had similar FBOM and algal biomass standing stocks at both scales (Figure 2.8 A-B). Patch ER rates at E2 in 2014 were the highest measured from all sites and also overestimated reach-scale rates more than any other site. FBOM densities varied substantially; with a difference of 60 g m^{-2} between the 25th and 75th percentiles within the 35 m reach. The highly variable abiotic conditions in this reach likely made it substantially harder to capture patch-scale variability in ER rates. However, patch-scale GPP rates overestimated reach-scale rates less substantially for this site. High canopy cover for this reach likely resulted in less patch-scale variability and rates that were more similar across scales. The structurally complex reaches from these examples may have required more patch-scale samples in order to fully capture variability within the reach. Overall, these reaches exemplify the importance of considering stream structural dynamics when scaling rates.

The importance of alternative substrata compartments

Besides weighting metabolic rates by the presence of their abiotic drivers, we also accounted for different substrata compartments in some reaches. The importance of substrata heterogeneity for biogeochemical rates has been well documented, especially for nitrogen (N)

cycling (Kemp and Dodds 2002, Findlay et al. 2011). Successful additive scaling of N uptake rates in prairie stream reaches has been attributed to characterizing available substrata compartments. O'Brien and Dodds (2008) compared ammonium uptake rates measured with experimental chambers and whole stream methods, and found that the direct comparison of uptake rates (i.e. uptake per unit area at each scale) was sufficient to approximate reach-scale rates from patch-scale measurements characterizing riffle and pool habitats. O'Brien et al. (2012) used patch and reach-scale isotopic ^{15}N experiments to quantify multiple N-cycling processes, finding that measurements at both scales agreed that the fate of N in that system was dominated by assimilation into the benthos rather than other processes. Both studies attribute reach-scale approximation with patch-scale measurements to the importance of characterizing all the available substrata compartments present in the stream.

Gravel and fine sediments made up 56-60% of the stream bed in E3 2013, and were the dominant substrata in all the other reaches. Cardinale et al. (2002) manipulated the presence of fine sediments in stream riffles while keeping median substrata size constant, finding that stream primary production and respiration rates responded immediately to additions of fine sediments. While gravel and fine sediments can be directly linked to biogeochemical rates, macrophyte presence could alter stream velocity (Dodds and Biggs 2002), resulting in changes to the immediate physical, chemical, and biological environment (Franklin et al. 2008). O`Brien et al. (2014) experimentally removed macrophytes from agriculture streams and found significant decreases in primary production relative to pre-removal rates. We measured metabolism rates from three compartments at E3 2013 due to the high areal cover of gravel and silt, macrophytes, and, leaf packs. The lack of high flow events and subsequent drought in the previous 2 years created ideal conditions for macrophyte growth and abundance (Franklin et al 2008), and the

deposition of leaf packs. If we had not accounted for these alternative compartments, estimated reach-scale rates would have been 50-60% lower, and patch rates would have underestimated reach rates even more than they already did. This is especially relevant for the E3 ambient reach, which had the most similar patch and reach-scale ER and GPP rates of all the reaches in this study.

There were still other compartments that we failed to account for that are likely important for accurate estimation of reach-scale biogeochemical rates. Filamentous algae consisted of 14 % and 18% of the benthic area in the E3 2013 ambient and removal reaches, respectively; however, we were not able capture their contribution to reach-scale metabolism. Kemp and Dodds (2002) found that filamentous algae contributed to nitrogen cycling in prairie streams. It's unclear to what magnitude filamentous algae may affect reach-scale metabolism rates. The hyporheic zone is another unmeasured compartment in this study that is also likely contributing to reach-scale ER rates (Findlay 1995). Hyporheic zones often contain water that is temporarily isolated from overlying flowing water (Mulholland et al. 1997), resulting in increased biogeochemical rates (Vervier et al. 1992, Boano et al. 2014). Sediment cores have been used to measure fine scale hyporheic conditions in large rivers (Xie et al. 2014). These methods could be translated to smaller headwater streams to determine hyporheic metabolism rates at small spatial scales.

In our study, long-term climatic trends significantly altered the available substrata in our reaches. Drought conditions led to the prolific abundance of macrophytes and leaf packs, while a flood in 2014 reduced the abundances of leaf packs, and almost completely removed macrophytes. The projected changes in climate in the Great Plains could lead to longer periods of drought with more intense flood events (Kunkel et al. 2008), which will likely be a major driver of the abundance of different biogeochemical compartments. Future estimates of metabolic rates

should account for all the present substrata compartments for estimating biogeochemical rates across scales. Our data suggests that climate effects cascade to stream metabolism and biogeochemistry as mediated hydrologic effects of flood and drought.

Considering other biogeochemical processes

Metabolism and N cycling are linked processes; both heterotrophic and autotrophic metabolism requires N (Webster et al. 2003). Helton et al. (2011) suggest the use of mechanistic models that consider both metabolism and N cycling rates together (with other ecologically relevant nutrients) when attempting to predict either process at the watershed scale. Functional relationships between nutrient concentration and uptake rates suggest that most of the reaches in Kings Creek are N limited (O'Brien and Dodds 2008). However, at the patch-scale, this may not always be the case. For example, patch-scale ammonium uptake rates were measured at E2 in 2014 from the same substrata baskets as the patch-scale metabolism measurements in this study (data not shown). Four of the ten patch measurements indicated a net production of ammonium, while the rest suggested net uptake over a 20 minute chamber incubation. The drastic differences in ammonium availability at this site are likely caused by the interaction of low stream velocity with the presence of N-excreting American Bison (*Bison bison)*, which frequented certain sections of this reach as indicated by the presence of bison trail crossings. Therefore, stoichiometric constraints (e.g. association of respiration and production with the magnitude of N- limitation) of metabolism rates were likely patch dependent. Using mechanistic modeling approaches that account for N cycling processes at this reach (and maybe others) may have improved predictions of reach-scale metabolic rates.

Conclusions

This study provides valuable insight to the abiotic and biotic effects that should be considered when up-scaling biogeochemical rates. While we focus on stream metabolic rates in prairie streams, the conceptual scaling framework from this study could be applied to any functional or structural aspect of any aquatic ecosystem by accounting for the local biotic and abiotic drivers. Our results suggest that additive scaling of habitat-weighted metabolic rates was not sufficient to predict reach-scale rates from patch scale measurements in a prairie stream. Patch-scale rates overestimated reach-scale rates for both GPP and ER after correcting for temperature and light conditions at the time of measurement, and metabolism rates from alternative substrata compartments. Additive scaling is one of the simplest approaches for predicting measurements from smaller to larger spatial scales; process or mechanistic based modeling approaches may provide better estimates of measurements at larger spatial scales. We used different methods to measure metabolic rates at each scale, which may have affected our ability to scale and should be considered in future studies. Overall, the best up-scaling method will accurately account for the major biotic and abiotic variables, which can be determined through observational or experimental measurements.

TABLES AND FIGURES

Table 2.1 Physical, chemical, and hydrologic parameters of the seven reaches used in this study. A= ambient consumer biomass, R= consumers removed

Watershed name	Units	Lower Kings (E3)			N1(E2)	K2a (E1)		
Lat		39.10004			39.08352	39.10006		
Long		-96.60959			-96.57723	-96.57436		
Burn frequency		Not burned			Annually	2 years		
Bison Present		No			Yes	No		
Year		2013		2014	2014	2013		2014
Consumer Treatment		A	R	A	A	A	R	A
Ambient NH_4^+	µg N L^{-1}	9		11	4	8		3
Ambient NO_3^-	µg N L^{-1}	4		NA	NA	BD		NA
Ambient SRP	µg P L^{-1}	9		NA	NA	8		NA
Dominant Substrata size (D50)	mm	18.7	NA	17.2	16.3	34.4	NA	21.7
Slope	degrees	0.22		0.22	0.58	0.56		0.56
Canopy Cover	%	61	48	67	81	58	53	58
Discharge	L s^{-1}	0.5	0.6	4.7	6.1	0.7	0.6	10.2
Base flow avg. width	m	3.76	2.34	4.02	1.52	1.78	2.10	3.08
Base flow avg. depth	m	0.23	0.10	0.03	0.12	0.03	0.08	0.03
Reach length	m	55	50	55	35	33	35	35

Table 2.2 Number of species, biomass of two most abundant species, and pre and post-treatment consumer biomass. *C. anomalum* = *Campostoma anomalum*, *P. erythrogaster* = *Phoxinus erythrogaster*, and *S. atromaculatus* = *Semotilus atromaculatus*. Additional information for individual consumer counts, biomass, and biomass for each reach is in Appendix B.

Reach	Highest biomass (g) by species, post-treatment		Area (m^{-2})	Total biomass (g m^{-2}), pre-treatment	Total biomass (g m^{-2}), post-treatment
E1 Ambient -3 species	*C. anomalum*	(20.9)	64	0.26	0.47
	P. erythrogaster	(4.3)			
E1 Removal -4 species	*P. erythrogaster*	(13.2)	105	0.14	0.28
	S. atromaculatus	(6.7)			
E3 Ambient -7 species	*C. anomalum*	(1363.3)	206	10.6	10.7
	P. erythrogaster	(684.4)			
E3 Removal -5 species	*P. erythrogaster*	(84.3)	117	5.2	1.7
	C. anomalum	(44.5)			

Table 2.3 Results of 2-way ANOVA between metabolism rates and standing stocks collected at E3 and E1 in 2013 with consumer removal and habitat type. Boldface indicates a significant ($p < 0.05$) effect. Groups with an asterisk are ln+1 transformed. df= degrees of freedom.

			E1		E3	
Response		df	F	*p*	F	*p*
GPP	Consumer	1,12	0.10*	0.758	0.597*	0.455
	Habitat	1,12	0.46	0.512	1.72	0.214
	Consumer x Habitat	1,12	0.0053	0.943	0.252	0.625
ER	Consumer	1,12	0.10	0.752	0.33	0.578
	Habitat	1,12	2.4	0.152	1.05	0.324
	Consumer x Habitat	1,12	0.511	0.491	0.09	0.767
FBOM	Consumer	1,12	1.049	0.329	2.82	0.118
	Habitat	1,12	0.070	0.797	5.41	**0.038**
	Consumer x Habitat	1,12	0.374	0.555	2.46	0.143
Chl *a*	Consumer	1,12	0.44	0.524	1.40	0.259
	Habitat	1,12	0.33	0.577	1.77	0.209
	Consumer x Habitat	1,12	1.88	0.199	0.547	0.474

Table 2.4 Reach-scale metabolic rates measured at the end of the 30 day incubation in 2013 and 2014. ER and GPP are in units of g m^{-2} d^{-1}. The range of estimated error for each rate is in parenthesis (see text for calculation of error estimates). A=ambient consumer standing stocks, R= consumers removed from the reach. Consumer manipulations did not occur in 2014.

Site	ER	GPP
E1A 13	0.09 (0-0.25)	1.67 (1.60-1.74)
E1R 13	3.71 (3.43-4.11)	3.32 (3.08-3.54)
E3A 13	2.94 (2.78-3.12)	1.46 (1.39-1.54)
E3R 13	1.71 (1.40-1.99)	1.12 (0.98-1.25)
E1 14	0.46 (0.44-0.49)	0.35 (0.33-0.36)
E2 14	3.55 (3.10-3.93)	1.37 (1.15-1.46)
E3 14	2.36 (2.15-2.60)	3.06 (2.94-3.18)

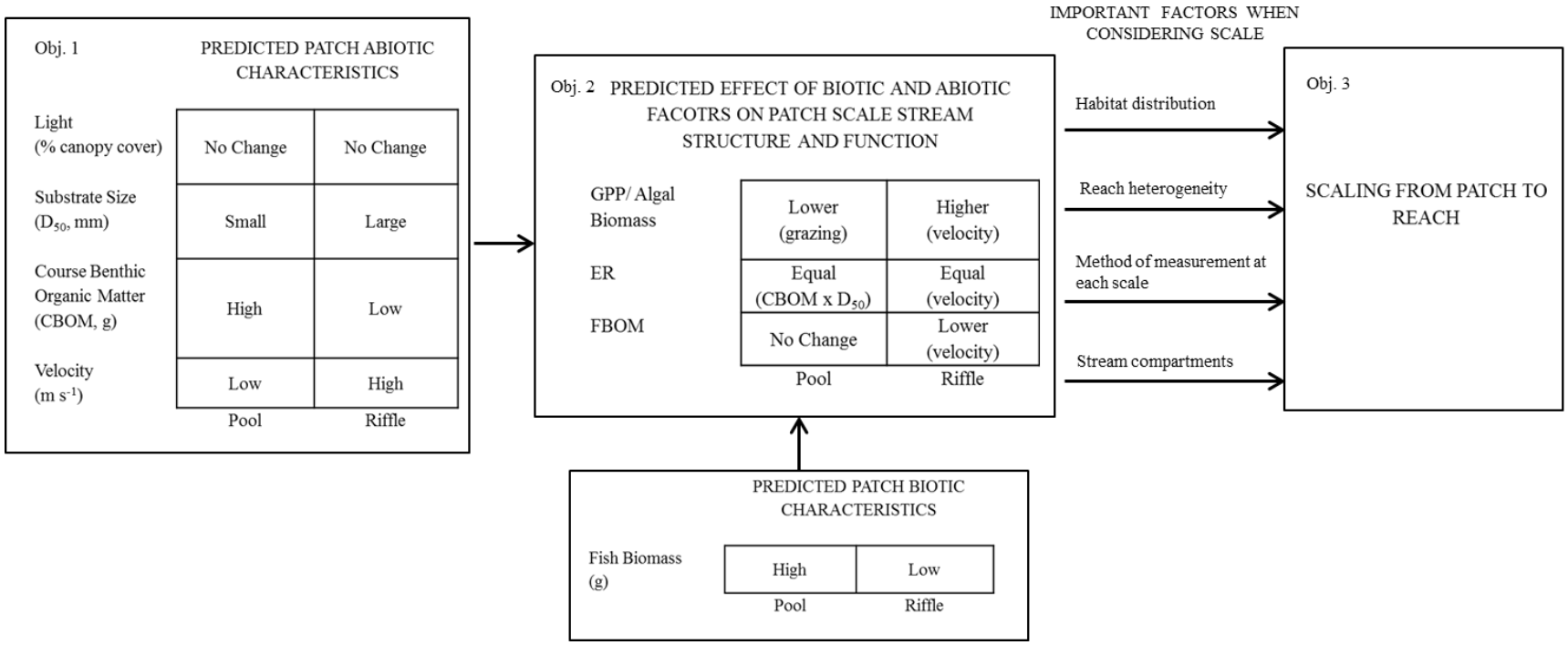

Figure 2.1 Conceptual framework for scaling metabolic rates. Predicted patch-scale biotic and abiotic characteristics within prairie stream pool and riffle habitats (Objective 1). Combined effect of these characteristics on patch-scale stream structure and function (Objective 2). We predict that we can accurately scale to larger spatial areas if we correctly characterize patch-scale biotic and abiotic effects and address other factors important to scaling (Objective 3).

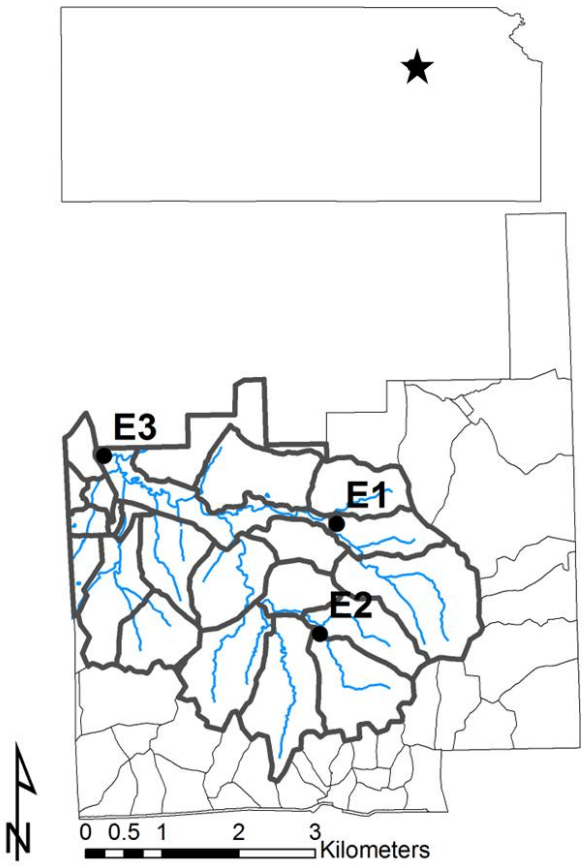

Figure 2.2 Location of sites within the Kings Creek watershed (bold outline) of Konza Prairie Biological Station (light outline). Measurements were taken at E1 and E3 in 2013 and 2014, and only in 2014 at E2.

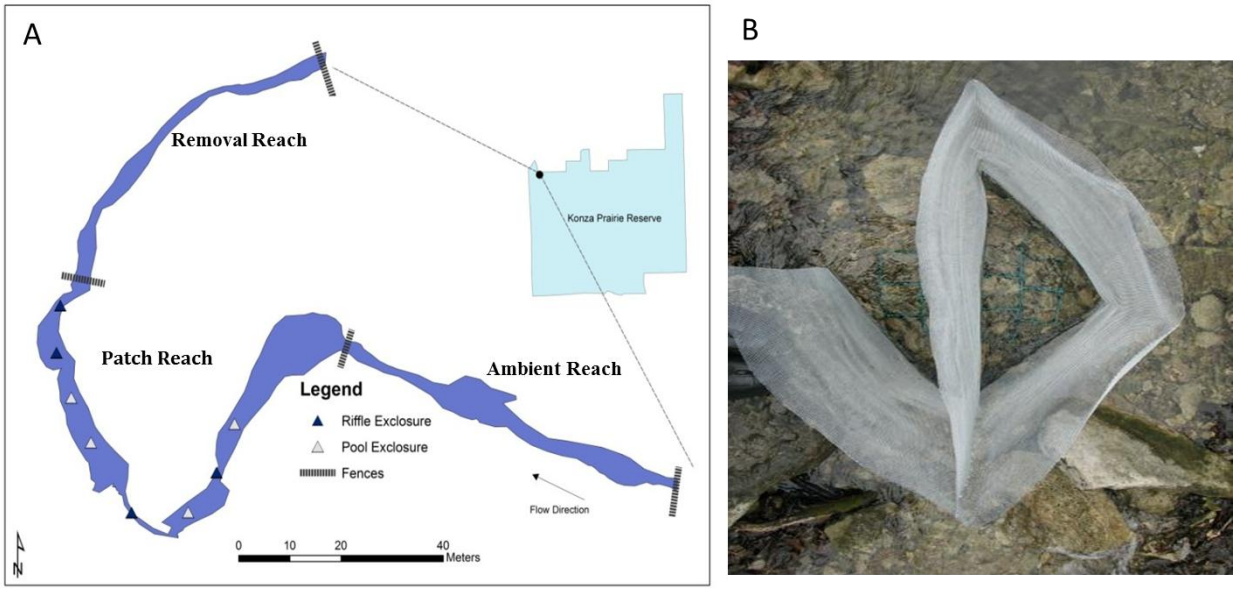

Figure 2.3 A: Experimental design and location of ambient, patch, and removal reaches at E3 in 2013 (E1 was set up in the same manner). B: Top-view of experimental exclosures used for patch-scale measurements. Stream flow is moving from the bottom to the top of the picture. Five substrata containers were placed in the open (left) and closed (right) side of the exclosure.

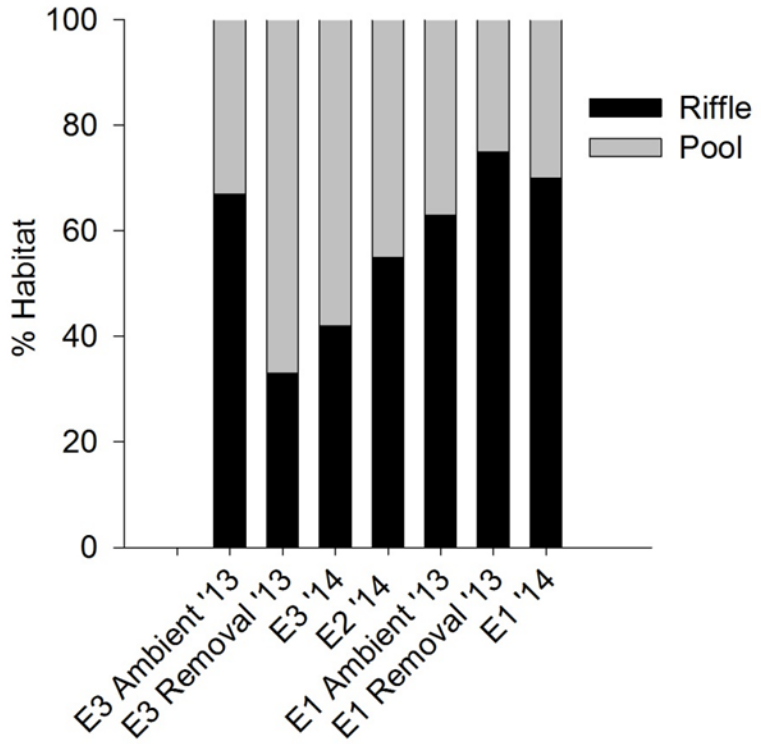

Figure 2.4 Stacked bar plot with the proportion of stream habitat from the various reaches used in this study. Reaches are aligned from lower in the watershed (E3) to higher in the watershed (E1/E2).

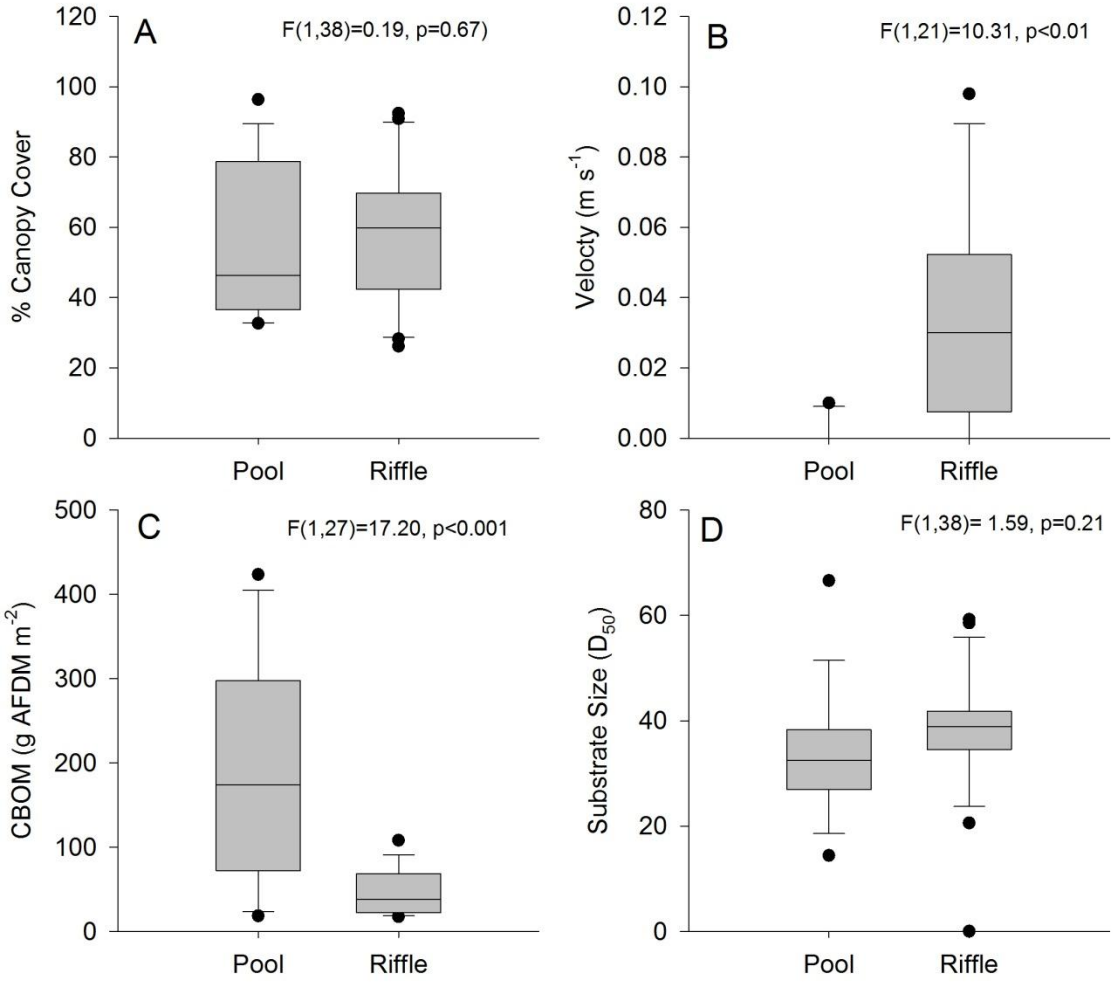

Figure 2.5 Boxplots of patch-scale abiotic characteristics (A=canopy cover, B=stream velocity, C= CBOM, D= substrata size) from pool and riffle transects from 2013 sites (both ambient and removal reaches from E1 and E3). Statistical outputs are from one-way ANOVA. Bars within boxes indicated median values. Upper and lower boundaries of boxes represent 25th and 75th quartiles, respectively. Error bars represent 10th and 90th percentiles, and solid circles represent those values outside the 10th and 90th percentiles.

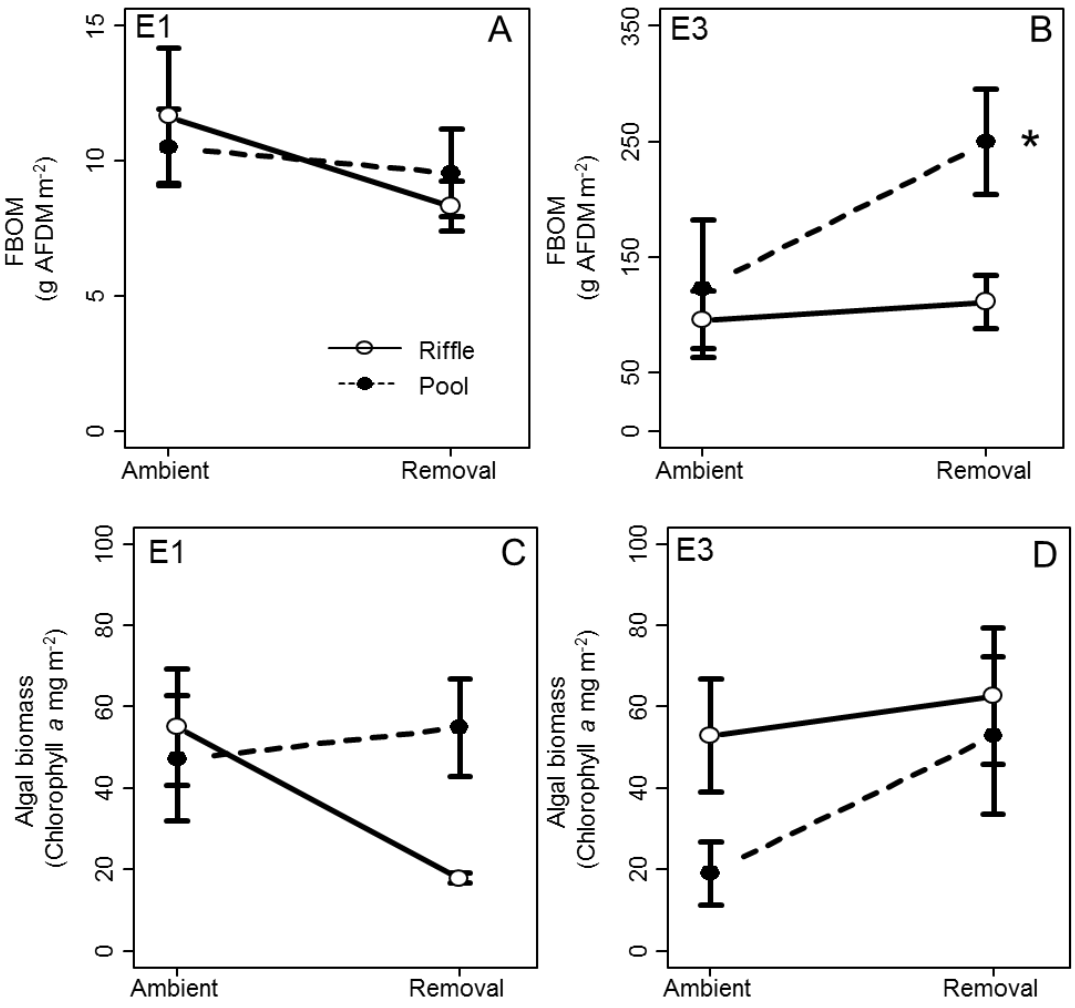

Figure 2.6 Comparisons of patch-scale differences between habitat and consumer removal for A: FBOM at E1. B: FBOM at E3. C: algal biomass at E1 and D: algal biomass at E3. Dashed lines and solid circles represent pool habitats, while solid lines and open circles represent riffle habitats. Error bar represent standard error of the mean. FBOM standing stocks were significantly lower after in riffles compared to pools ($p<0.05$) and marginally increased after consumer removal ($p=0.11$) at E3 (panel B). See Table 2.3 for output of statistical test.

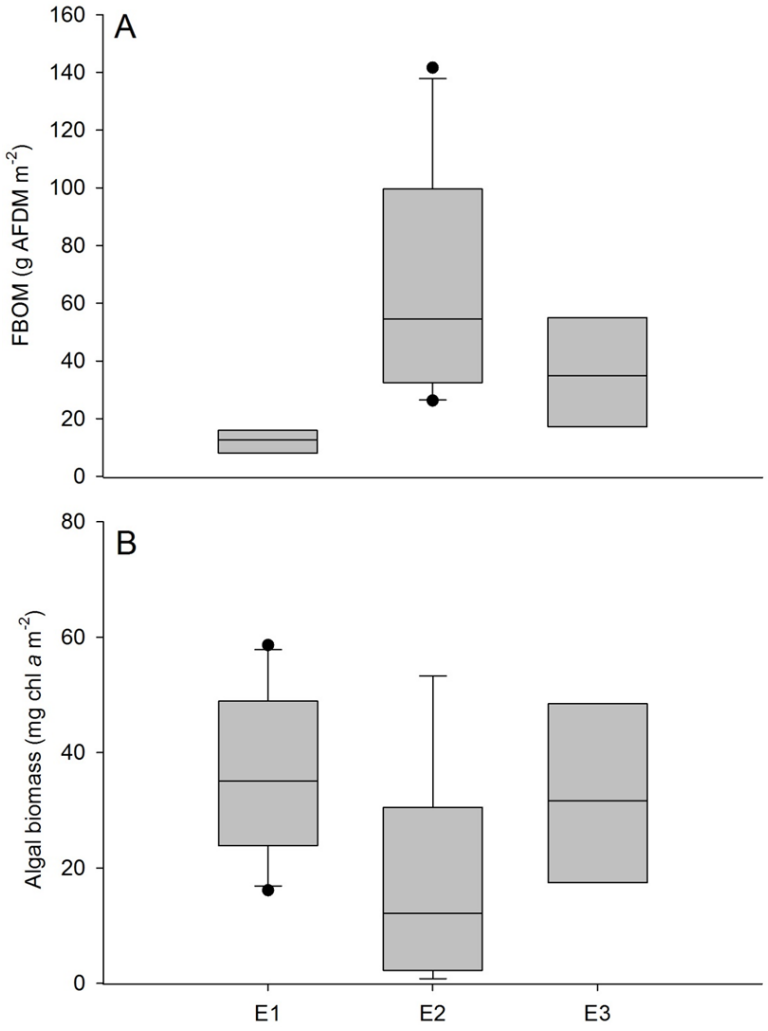

Figure 2.7 Standing stocks of algal biomass (A) and FBOM (B) collected from reach transects (n=10) across the three sites measured in 2014. Bars within boxes indicated median values. Upper and lower boundaries of boxes represent 25th and 75th quartiles, respectively. Error bars represent 10th and 90th percentiles, and solid circles represent those values outside the 10th and 90th percentiles

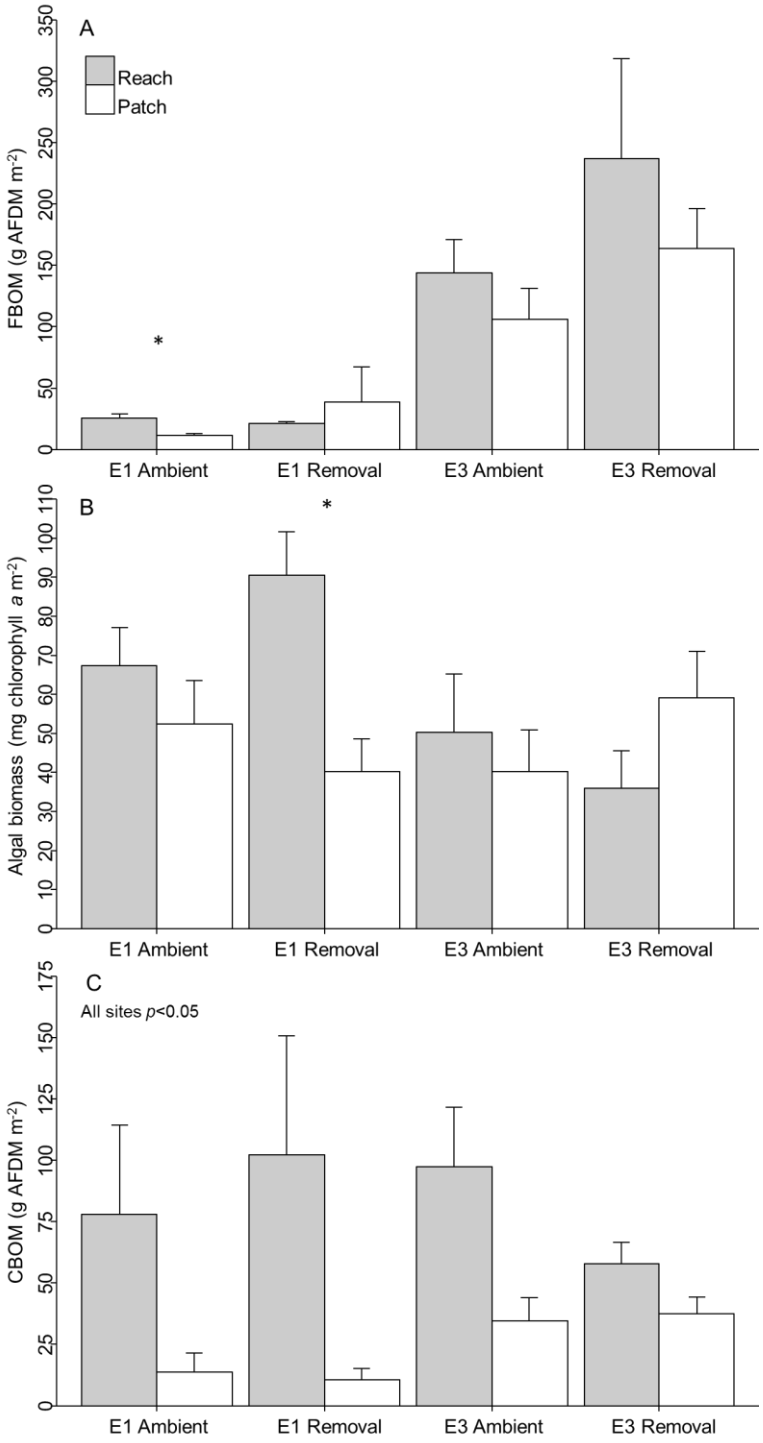

Figure 2.8 Comparisons of mean (with standard error) reach and patch-scale standing stocks of FBOM (A), algal biomass (B) and CBOM (C) from 2013 sites. Gray bars represent reaches where reach-scale measurements were made, and open bars represent reaches where patch-scale measurements were made. Asterisks represent statistical differences ($p<0.05$) between patch and reach-scale measurements.

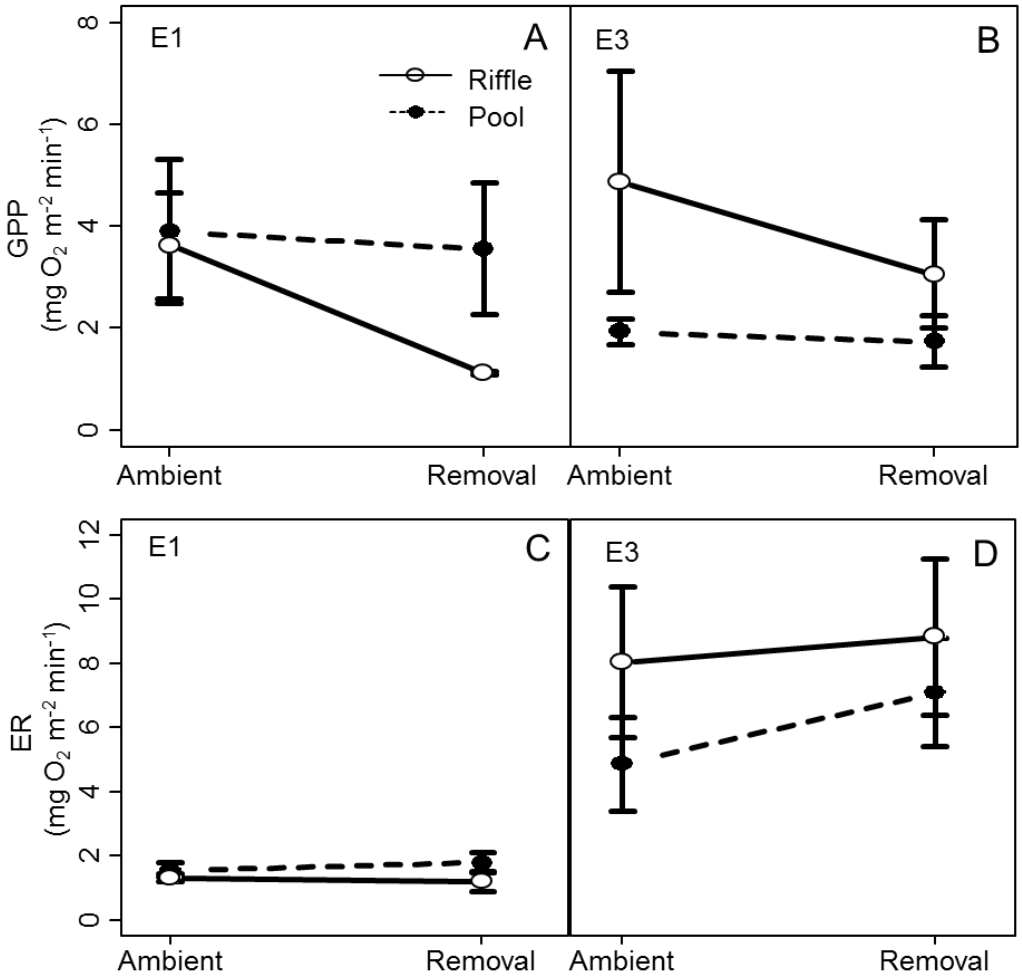

Figure 2.9 Comparisons of patch-scale metabolic rates between habitat and consumer removal for A: GPP at E1. B: GPP at E3. C: ER at E1 and D: ER at E3. Dashed lines and solid circles represent pool habitats, while solid lines and open circles represent riffle habitats. Error bars represent standard error of the mean. There were no significant relationships ($p < 0.05$) between GPP or ER between habitat or consumer treatments using a two-way ANOVA. See Table 2.3 for output of statistical tests.

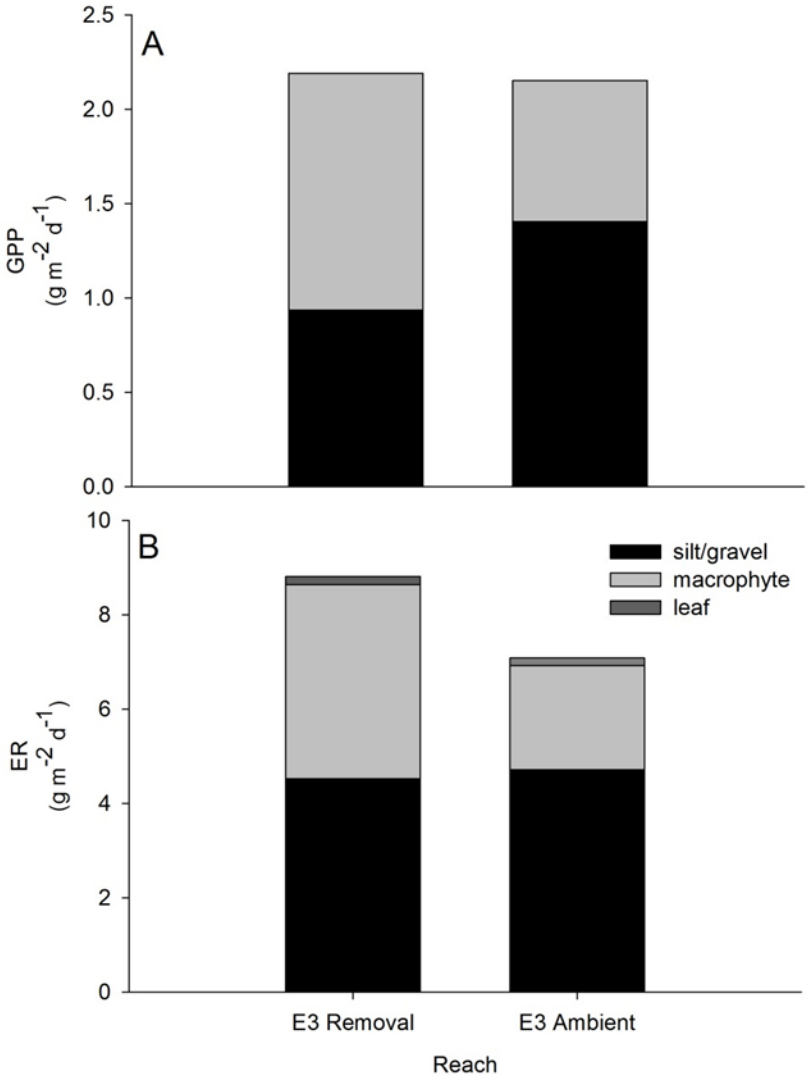

Figure 2.10 Stacked bar graph of contributions of various compartments to patch-scale GPP (A) and ER (B) rates. Each rate is weighted by the proportion of that compartment within the reach (see equation 5). All compartments were measured with experimental chambers. Silt/gravel samples were measured from incubated baskets, while macrophytes and leaf packs were taken directly from the stream.

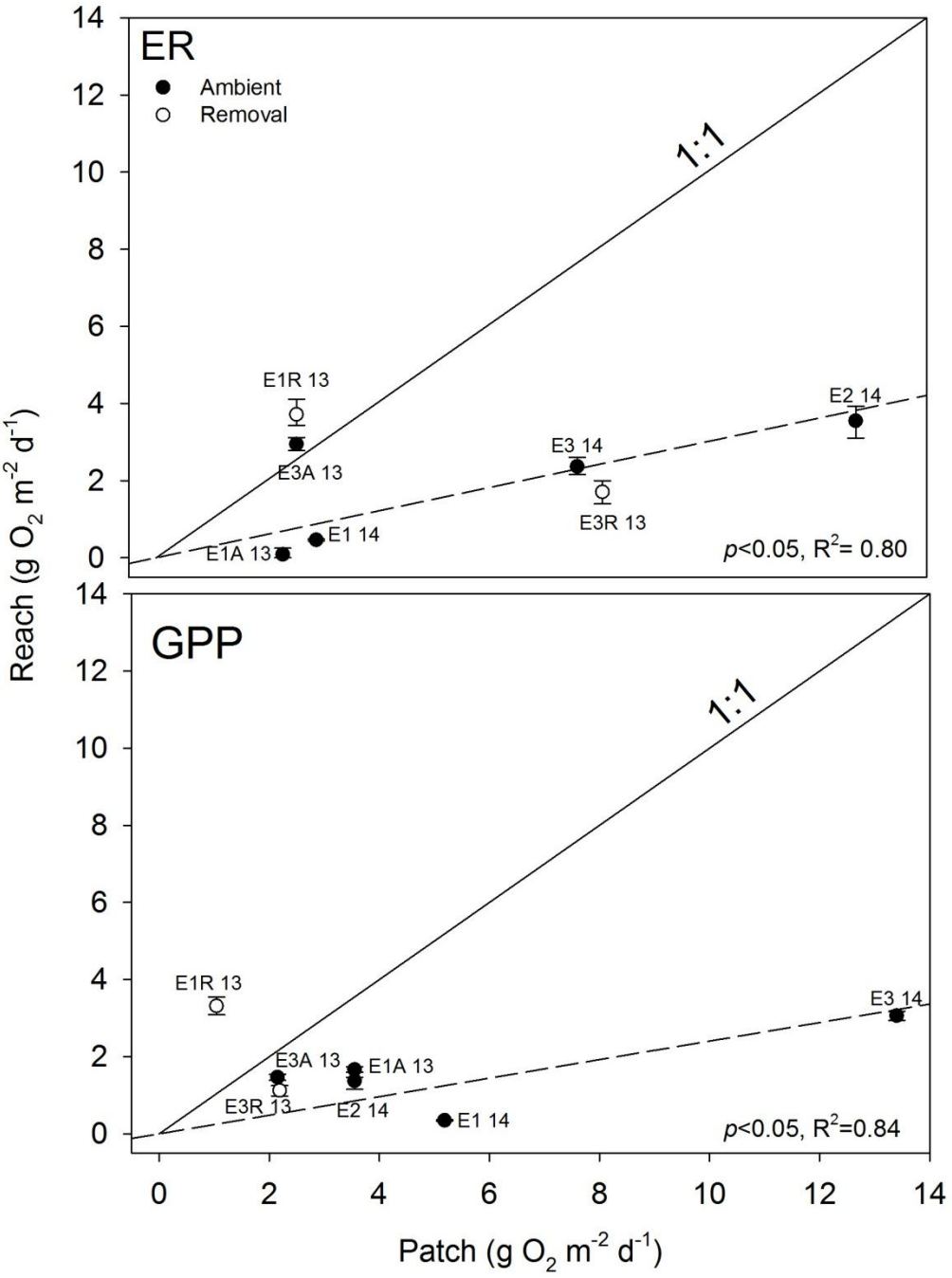

Figure 2.11 Comparison of patch and reach-scale ER (top) and GPP (bottom) rates with 1:1 reference line. Error estimates are present for only reach rates. Filled in circles represent measurements with ambient consumer biomass, while open circles are from removal measurements. Linear regression represents reaches with ambient consumer biomass.

Chapter 3 - Biotic and abiotic controls of patch-scale biogeochemical fluxes across a prairie stream network.

Abstract

Biogeochemical fluxes within streams can vary with local environmental conditions as well as variation in the distribution of fishes. We used multiple path analyses (n=3) to elucidate direct and indirect effects of fish presence and other abiotic factors (fine benthic organic matter (FBOM), algal biomass, canopy cover, substrata size, wetted width, and watershed location) on fine scale (300 cm^2) benthic rates of ecosystem respiration (ER), gross primary production (GPP), and ammonium uptake in a prairie stream. Indirect effects of fish on biogeochemical rates mediated through FBOM and algal biomass were also tested in each model. Biogeochemical rates of stream-equilibrated substrata (after a 30 day stream incubation, n=49) were quantified by monitoring fluxes of dissolved O_2 (in light and dark) and ammonium inside sealed acrylic chambers with internal circulation systems. Biotic effects were determined by comparing substrata exposed to fish to those with fish excluded. Total model-explained variance was highest for ER (65% as R^2) and lowest for GPP and ammonium uptake (38%). Location of measurements in the watershed was not a significant factor for any of the biogeochemical rates; however, the model suggested location strongly influenced FBOM standing stocks. Fish presence directly increased ammonium uptake and GPP, while all rates were indirectly affected through changes in either FBOM and /or algal biomass. Significant paths of abiotic factors varied with each model; however, substrate size was important for all rates. Univariate analyses of a subset of data and path models both agree that biotic and abiotic factors interact to affect ammonium uptake, while GPP and ER rates are likely driven primarily by abiotic factors.

Introduction

Quantifying stream biogeochemical fluxes at patch-scales (i.e. benthic area measured in cm^2) can be useful for understanding fluxes across larger spatial scales (Pringle et al. 1988). Patch-scale dynamics are especially vital to headwater streams, where hydrologic, geomorphic, and biological processes are spatially and temporally variable (Gomi, Sidle, and Richardson 2002). Measurements at patch-scales are often easy to replicate compared to similar measurements at larger scales, and allow for the capturing of spatial variability within a nested scale of interest. For example, Sakamaki and Richardson (2013) discovered non-linear thresholds for biogeochemical proxies across bank-full width transects within a forested stream network, highlighting the potential for scale-dependency of functional stream characteristics across varying abiotic conditions.

Prairie streams exhibit an exceptional amount of abiotic spatial and temporal variability (Larson et al. 2013a). This has been attributed to intense climatic (flood and drought), fire, and terrestrial grazing disturbances (Dodds et al. 1996, Larson et al. 2013b). Abiotic stream characteristics can often dictate the presence and abundance of prairie stream fishes (Martin et al. 2013, Troia and Gido 2014), while fishes can have reciprocal effects on both structural and functional components of their environment (Berke 2010). The Central Stoneroller (*Campostoma anomalum*), a common grazer in prairie streams, can be directly responsible for reductions in attached algal height (Power and Matthews 1983) and periphyton biomass (Gelwick and Matthews 1992, Gelwick and Matthews 1997). Besides prairie streams, grazing effects of fish have also been identified in tropical (Flecker 2002), coastal forest (Power 1990), and temperate forest (Abe et al. 2007) streams.

The effects of fish on stream structural components have been well characterized; however, they are often context specific (Vanni 2010). For example, Southern Redbelly Dace

(*Phoxinus erythrogaster*) can decrease algal and macroinvertebrate biomass during flood recovery (Murdock et al. 2011); while periphyton communities respond to precipitation disturbances better with the presence of grazers (Bertrand et al. 2009). Murdock et al. (2010) experimentally manipulated the presence of fish during stream re-wetting following drought conditions, finding that fish presence altered both stream structure and function. Alternatively, fishes can increase algal biomass due to nutrient loading from excretion (Kohler et al. 2011) by increasing nitrogen availability in nutrient limited streams (McIntyre et al. 2008). Fish effects on both structure and function of streams can often be temporally variable, changing annually due to droughts or floods (Pringle and Hamazaki 1997, Power et al. 2008), seasonally due to changes in temperature (Bengston et al. 2008), or in a matter of weeks following recovery from a disturbance (Murdock et al. 2010).

The effect fishes have on ecosystem function is less well documented. Many studies investigating the effect of fishes on streams focus on structural rather than functional components (Reisinger et al. 2011), even though fish could influence stream function through multiple pathways. Biogeochemical fluxes can be affected by grazing, bioturbation (for benthic-dwelling species and grazers), and increased nutrient loads from excretion (Berke 2010). Fish removal can affect ecosystem respiration (ER) and ammonium uptake in streams (Murdock et al. 2010); however, fish removal didn't affect gross primary production (GPP; Bengston et al. 2008) or denitrification rates in experimental mesocosms (Reisinger et al. 2011).

Multiple studies have investigated abiotic controls on a variety of biogeochemical fluxes. A large contribution to the field was the result of the Lotic Intersite Nitrogen experiments (LINX collaborators 2014). This series of experiments was conducted within 72 streams across the contiguous U.S. and Puerto Rico, and provided insight to the controls of reach-scale ecosystem

rates across a broad spectrum of biomes, land uses, and environmental conditions. For example, Bernot et al. (2010) used structural equation modeling on LINX II data to elucidate direct and indirect effects of abiotic stream conditions on stream metabolism. Their results suggest that land-use, nutrients, and light affect GPP, while organic matter content influenced ER. While researchers cannot assume that these associations will be representative of any given region, the results of the LINX studies can be used as a basis for testing these drivers in a specific watershed.

Structural equation modeling (referred to as path analysis in this paper) is useful tool for evaluating multivariate hypotheses under a flexible statistical modeling framework (Grace and Bollen 2008). The method has an advantage over multiple regression approaches in that it allows more complex interactions among variables to be evaluated, including paths of influence. Path analysis is fairly underutilized in ecology, but has been suggested as a possible tool to advance the ecological sciences (Belovsky et al. 2004). We investigated the biotic and abiotic controls of patch-scale (i.e. cm^2) metabolism (ER and GPP) and ammonium uptake rates on Konza Prairie Biological Station (KPBS) using path analyses, and compared the results to a traditional univariate analysis on a subset of the data. The goals of this study are: 1) determine the direct and indirect effects of stream fish presence on biogeochemical rates at the patch-scale, and 2) simultaneously evaluate the importance of abiotic stream characteristics in explaining variation in rates. Initial predicted models were built using data from previous studies completed within the Kings Creek watershed at KPBS, as well as results of the LINX I and II experiments (see Figure 3.2 for predictions and citations).

Methods

Path Analyses

We identified five biotic and abiotic continuous variables to include in the original model that have been shown to individually affect biogeochemical rates. Canopy cover is used as a surrogate of light availability, which has been shown to be an important driver of both ER (Riley and Dodds 2012) and GPP (Mulholland et al. 2001, Riley and Dodds 2013). Median substrata size (D_{50}) can represent available microbial habitats and pore space, both of which are important for biogeochemical rates (Kemp and Dodds 2001, Kemp and Dodds 2002). Wetted width is used as quantitative measurement of stream habitat, which can affect ammonium uptake rates (O`Brien and Dodds 2008). We predict fish might directly affect both FBOM (Gido and Jackson 2010) and algal biomass (Murdock et al. 2011, Kohler et al 2011) through bioturbation and grazing, respectively (Berke 2010). Indirect effects of fish on rates will likely be mediated through these variables, which have been shown to also affect benthic metabolism (Bernot et al. 2010). Here we include algal biomass within the general term "abiotic factors" for simplicity of discussion of basal factors driving GPP and serving as a resource for higher trophic factors, while recognizing this is actually a biotic driver. Data for these analyses was collected across two sites (referred to as 'E1' and 'E3') in the Kings Creek watershed (Figure 3.1). The two reaches differ substantially in geomorphology and flow variability, where E1 is a 2^{nd} order spring fed headwater reach with variable flow and is prone to desiccation, while E3 is a 5^{th} order reach that is heavily incised with permanent flow. Categorical variables for each rate in these analyses include the presence or absence of fish ('fish'), site (E1 and E3), and year (2013 or 2014).

We used the *lavaan* and *lavaan.survey* packages (Rossel 2012) in R (version 2.15.1; R Project for Statistical Computing, Vienna, Austria) to conduct path analyses (n=3) for each

biogeochemical rate based on a standard hypothesized path model (Figure 3.2). The 'sem' function was used to fit the hypothesized model for each rate. Model fit was assessed by a chi-square test comparing the observed and fitted covariance matrices. A non-significant test indicates statistically similar matrices, and a fit model. Each initially fit model was then analyzed with the 'lavaan.survey' function, which provided similar output statistics as the 'sem' function, while informing the model that data was collected across multiple years. This was done to control for variation between years without adding year as a variable in each model. The Satorra-Bentler correction was applied to model output indices in order to correct for the non-normality of the database.

We tested the importance of the paths from Fish → Rate and Site→ Rate in each model (Figure 3.2) beyond that of individual model path p-values since these relationships are of primary interest, and backed by limited or confounding data. All other paths originally in the hypothesized model were included in the final model since previous research suggests a relationship could exist, even if the path wasn't significant. The importance of fish and site paths on each rate was tested by comparing overall model fit (model Chi –square and AIC), covariance matrix residuals, and modification indices of models with and without these paths. When there was no effect on the model fit after dropping either path, it was removed.

Data selection

Data used in these analyses are introduced in detail in M Trentman Thesis Chapter 2. Measurements of biogeochemical rates (GPP, ER, ammonium uptake) standing stocks of fine benthic organic matter (FBOM) and algal biomass (chl *a*), as well as other environmental conditions were measured from individual patches (n=49) spread across two sites on KPBS in late spring of 2013 and 2014 (Figure 3.1). All rates and standing stocks were measured after a

30-day stream incubation from plastic containers (i.e. strawberry baskets; 11.4 cm x 9.5cm x 6.6 cm) with an effective mesh size of 1cm filled with substrata representative of the stream. Approximately half of the total containers (n=22, 2013 only; Table 3.1) were subject to an experimental removal of fish with a constructed exclosure device, which prevented fish from accessing baskets via a fine hardware mesh (1 cm mesh interval). Fish exclusions were initially replicated in 2014, but exclosure equipment was lost in a flood. The loss of this equipment and subsequent samples, and the naturally low biomass of fish at E1 led to an unbalanced distribution of patch samples across site, year, and fish manipulation variables (Table 3.1). We recognize that the unbalanced distribution of samples for this experiment results in a non-ideal data structure, and therefore results from path models should be taken with caution.

All biogeochemical rates were measured from three incubated baskets from each exclosure or transect in sealed acrylic chambers containing an internal propeller circulation system (Rüegg et al. *In Press*). Incubated containers were carefully removed from the stream and placed in the chamber, filled with stream water, and sealed. We monitored changes in oxygen (in light and dark) and ammonium across a 70 minute incubation period for each sample. The measurements were used to calculate ER, GPP, and ammonium uptake rates (see Rüegg et al. *In Press* for a detailed explanation of chambers and methods used for measuring and calculating biogeochemical rates). Both ER and GPP rates were corrected to 20° C and 300 PAR (Photosynthetically Active Radiation; GPP only), since chamber measurements were taken at varying times throughout the day. Temperature corrections were made using published equations (Parkhill and Gulliver 1999), while light corrections were made using a photosynthesis-irradiance curve created from separate chamber patch-scale measurements under varying light intensities (Appendix A).

Standing stocks of FBOM and chl *a* were measured using standard methods on the same day from a single container adjacent to those used for biogeochemical rate estimates (see M Trentman thesis Chapter 2 for explanation of methods). Other abiotic variables used in each path analyses include: wetted width, canopy cover, and median substrata size (D_{50}), which were all measured prior to patch container installation. Canopy cover was measured using a spherical densiometer, and D_{50} was calculated as the median substrata size of 10-20 particles collected at each transect.

Univariate tests

A subset of experimental data from the dataset used for path analyses was analyzed using univariate tests. Two-way ANOVAs (one for each rate and both standing stocks, n=5) were used to determine the effect of experimental removal of fish from exclosures in 2013 and transect habitat (i.e. riffle or pool). We included habitat in this analyses because fish presence may interact with abiotic stream characteristics to affect stream structure and function (M Trentman thesis Chapter 2). Non-normal response variables, as indicated through the Shapiro-Wilk Normality Test were natural log transformed. All analyses were conducted in R (version 2.15.1).

Results

Patch-scale temperature and light standardized metabolism rates varied spatially and temporally. GPP values ranged between 1-13 mg O_2 m^{-2} min^{-1} in 2013 and 3-17 mg O_2 m^{-2} min^{-1} in 2014 (Figure 3.3 A). ER rates ranged between 1-15 mg O_2 m^{-2} min^{-1} in 2013 and 1-9 mg O_2 m^{-2} min^{-1} in 2014. (Figure 3.3 B). Ammonium uptake rates ranged between 7-621 µg m^{-2} min^{-1} in 2013 and 60-341 µg m^{-2} min^{-1} in 2014, (Figure 3.3 C). Standing stocks of FBOM were highest at E3 in 2013. Total standing stocks ranging from 4.8 to 330.2 g AFDM m^{-2} (Figure 3.4 A). Total chl *a* ranged between 0.2-122.4 g m^{-2} (Figure 3.4 B). Univariate testing suggested a marginally

significant increase in ammonium uptake rates after fish removal at E3 (ANOVA, F=4.46, p=0.056; Figure 3.3 F). There wasn't a statistically significant difference in metabolism rates or standing stocks after fish removal (or interaction of fish removal with stream habitat) at either site (Figure 3.3 D,E; 3.4 C-D. Wetted widths across transects ranged from 0.75 m to 8.3 m, and were generally wider downstream. Canopy cover ranged from 25.9% to 89.9% across all transects. Median substrata size (D_{50}) ranged between 1.2-122.4 mm.

Fish biomass were measured in reaches above and below the exclosures at each site as a part of a separate experiment (Trentman thesis Chapter 2), but not directly in the reach with exclosures. At E3, fish biomass ranged between 5.2-10.7 g m^{-2} around the exclosures, while E1 fish biomass were much lower, ranging between 0.14-0.26 g m^{-2}. Both sites were dominated by *C. anomalum* and *P. erythrogaster* (Appendix B).

Model modifications

Path models based off the initial hypothesized model did not adequately fit the data for any of the biogeochemical rates (i.e. Chi-square $p < 0.05$). The residuals between the fit and measured covariance matrices suggested a relationship between the Site → FBOM path. Furthermore, the modification index for this relationship was greater than 5, suggesting that a larger amount of residual variation would be explained with the addition of this path (Rossell 2012). Our data followed the expected pattern of increasing FBOM from upstream to downstream (Figure 3.4 A), so it was appropriate to add this path. All path models adequately fit the data using this new model (Table 3.2, columns 3-5).

Model modification tests suggested dropping the Site→Rate path in all three models. This path was not significant in any of the models, and removal of this path did not significantly alter total model-fit statistics or AIC values. The Fish→Rate path was dropped from the ER

model. This path was not significant in the initially fit model, and removal of the path did not significantly alter total model-fit statistics or AIC values. Removal of this path from the GPP and ammonium uptake models resulted in a model that no longer fit the data. Examination of residual correlations and modification indices suggested that this path should be re-introduced, so this path was included in the final model for these rates. The final model for ER explained 65% of the total variance (as R^2), while the model for GPP and ammonium uptake each explained 38% (Figures 3.5-3.7). Fit statistics for the final models are summarized in Table 3.2 (columns 6-8).

Direct and indirect effects of fish removal

All path models suggested that fish removal increased chl *a* and FBOM standing stocks (Figure 3.5-3.7). The interpretation of the path coefficients for these paths varied based upon the presence of other predictor variables acting on either standing stock. Since no other predictors are acting on chl *a*, the unstandardized coefficient represents the slope of the regression between fish presence and chl *a*. Alternatively, FBOM has multiple predictors acting on it (fish and site); therefore the unstandardized coefficient suggests that fish removal increases FBOM when the effect of site is held constant. All path models also suggested an increase in FBOM standing stocks moving from upstream to downstream in the network after controlling for the effect of fish.

As noted above, the fitted path models did not suggest a direct effect of fish removal on ER (Figure 3.5); however, both ammonium uptake and GPP models suggested that fish removal resulted in decreases in these rates (Figures 3.5-3.7). The unstandardized coefficient for ammonium uptake (-88.24) was much higher than GPP (-3.92); however, the ammonium uptake path was marginally significant. Indirect effects of fish removal on all rates are mediated through significant paths from chl *a*/FBOM → Rate. The path between FBOM → Rate was significant

for all models, with positive relationships for ER and ammonium uptake, and a negative relationship for GPP. Standardized coefficients were highest for these paths in ER (0.58), intermediate for ammonium uptake (0.36) and lowest for GPP (0.22). Similarly, in the GPP model the unstandardized coefficient was very low (-0.01). Standardized coefficients for the path from chl *a*→ Rate were low in both ER and ammonium uptake models (<0.17), while the path was not significant for GPP.

Direct effects of abiotic factors

Median substrata size was the only environmental variable to be significant across all models. This relationship was positive for GPP and ammonium uptake and negative for ER. Canopy cover was not a significant variable in any model. There was significant positive relationship between width→Rate for both GPP and ER.

Discussion

Data Structure Issues

The unbalanced layout of data used in the path models was caused by the absence of experimental exclusion of fish in 2014, which were not completed due to the loss of exclosure equipment in a flood early in the 2014 field season. Despite this, the results of path analyses are subject to criticism and it is for this reason that we compared the outcomes of path models with a subset of experimental data tested with standard univariate methods. The results of path analyses matched univariate tests for most of the possible effects of fish on stream structure and function (see below). We identify fish effects to be most likely when both path models and univariate models agree, and less likely when only one of the two methods suggest fish effects.

Fish presence and stream structure

The presence or absence of fish in this study had significant effects on stream structure and function. Results of fish effects on stream structural components using path analyses were consistent with our prediction that there would be higher FBOM and chl *a* when fish were not present (Figure 3.2). These effects were most evident for FBOM, with higher standing stocks when fish were absent using both path analysis (as unstandardized path coefficient, Figure 3.5) and univariate tests (Figure 3.4 C). Both fish and site variables accounted for 45% of the variation (as R^2) in FBOM standing stocks in path models, with the site→ FBOM having an unstandardized path coefficient three times higher than the fish → FBOM path (Figure 3.5). This suggests that location had a more substantial influence on FBOM standing stocks than fish presence. FBOM standing stocks at E3 in 2013 ranged between 60-400 g m^{-2}, which was dramatically higher than any measurement the next year, or any value at E1 across both years (Figure 3.4 A). The high values at this site and year were likely driven by a drought and subsequent lack of high flow events in the previous 2 years. The densities of FBOM were dramatically reduced in 2014 following a 2.5-year flood prior to measurements for the 2014 season. The results of this study suggest that watershed location is the primary driver of FBOM standing stocks, but fish likely also play a role in the distribution of FBOM.

Results from path analyses suggest that chl *a* was significantly higher when fish were absent (Figure 3.5). Non-significant results were found using univariate analysis at both sites; however, there was a similar non-significant increase after experimental consumer removal at E3 (Figure 3.4D). Previous studies suggest that fish effects on chl *a* may be specific to individual species of grazers. A study in temperate stream pools suggested that the presence of *C. Anomalum* resulted in lower algal biomass (Gelwick and Matthews 1992), while an experiment in stream mesocosms did not detect an effect of Southern Redbelly Dace (*P. erythogaster*) on

algal biomass (Bertrand and Gido 2007), despite algae being a major part of both species` diet. The E3 exclosures were surrounded by reaches dominated by a mixture of *C. Anomalum* and *P. erythogaster*; however, fish biomass were higher for *P. erythogaster* (3.3-4.2 g m^{-2}) and lower for *C. Anomalum* (0.7-1.0 g m^{-2}, data not shown). This suggests that the marginal to non-existent effect of fish on chl *a* found in this study may be due to the relatively low biomass of *C. Anomalum*. Future studies could evaluate species specific (with varying biomass of fish biomass) and interactive tests of different stream grazers on algal biomass.

Fish effects on FBOM and algal biomass and periphyton biomass have been well documented during recovery from both high flow events and stream re-wetting after desiccation. A meta-analysis by Gido et al. (2010) suggests that consumer effects on organic matter are more likely to occur within 2 weeks of a major event and effects on algal biomass occurring more than 4 weeks after a major event. Stream conditions in 2013 were not affected by a high flow event, but discharge in the reaches used in this study were dramatically lower than a typical year (Walter Dodds, personal communication), and were decreasing during the length of the experiment in 2013. We found fish effects on FBOM and no effects on algal biomass after a 4 week incubation and on-going stream draw-down using experimental univariate analyses. The results of this study add to the already abundant literature showing interactive effects of climate and fish on stream structure.

Fish presence and biogeochemical rates

The absence of fish did not directly affect ER rates from either path analysis or univariate statistical tests; the path model for ER was the only model where removal of the Fish→Rate path did not alter model fit statistics (Figure 3.5). This model did identify indirect effects of fish on ER mediated through FBOM and chl *a* standing stocks. The unstandardized coefficient for the

FBOM→ER path was relatively low (0.03), suggesting a minimal increase of 0.03 mg m^{-2} min^{-1} with every unit increase in FBOM after controlling for the effect of fish presence. The standardized coefficient for this path was relatively high (0.58), suggesting that this relationship is one of the strongest relative to all the other variables in the model. Fish also indirectly affected ER through chl *a*; however, both standardized and unstandardized coefficients for this path were low suggesting that indirect effects are minimal and less likely drivers of ER than indirect effects mediated through FBOM.

The variation of ER rates between sites and years followed similar trends as FBOM, with higher rates and variation during low-flow conditions at E3 in 2013 (Figure 3.3 B and 3.4 A). Acuña et al. (2005) found increasing organic matter and FBOM build-up with high ER rates in isolated pools during low flow events. Our streams were connected throughout the extent of the incubation period but residence times in the larger pools were very high (as measured with NaBr releases, data not shown) very nearly mimicking conditions in an isolated pool, which might explain the occurrence of high FBOM standing stocks and ER rates. Murdock et al. (2010) found marginally lower ER after fish removal in prairie streams following drought recovery; however, these effects were temporally variable and FBOM values were not reported. The use of path analysis identified an extra level of effects (i.e. effects mediated through FBOM), which may not have been identified using standard statistical tests.

Fish indirectly affected ammonium uptake through both FBOM and chl *a* standing stocks. The unstandardized coefficients were relatively similar in value but different in direction, suggesting similar changes in ammonium uptake with a single unit positive change in FBOM or negative change in chl *a* (Figure 3.6). The standardized coefficient for FBOM was double that of chl *a*, suggesting that FBOM is a more important driver of ammonium uptake rates. Both path

models and univariate tests suggested a direct decrease in ammonium uptake rates after fish removal (Figures 3.6, 3.3 F). Unstandardized path coefficients suggest a decrease in uptake rates of 90 µg m^{-2} min^{-1}, while experimental removal of fish at E3 in 2013 lowered rates by ~180 µg m^{-2} min^{-1}.

Murdock et al. (2010) found a significant increase in ammonium uptake rates following removal of fish during a stream recovery from desiccation; however, effects on ammonium uptake were temporally variable. We found differing fish effects on ammonium uptake in this study using path analysis and a mixture of data collected from streams exhibiting stream draw-down in 2013 and recovering from a flood in 2014. Experimental fish manipulations in this study specifically identified fish effects in a stream reach exhibiting draw-down alone. Gomez et al. (2012) found that intermittent Mediterranean streams moving toward desiccation had significantly higher nitrate and lower ammonium concentrations. Similarly, prairie streams can switch from positive to negative net primary production (NPP; i.e. net heterotrophy) during stream draw-down (Dodds et al. 1996). Fish effects during stream draw-down could enhance the alteration of nutrient availability through excretion (with a lower volume of water) and more time spent closer to the benthos. Our study is the first to observe fish effects on ammonium uptake rates during stream draw-down in Great Plains streams; however, it is documented that stream draw-down can alter fish community assemblages (Perkin et al. 2015) and fish habitat refuge (Falke et al. 2011). Most studies investigating stream draw-down and desiccation are concentrated in intermittent Mediterranean climate streams (Bernal et al. 2013). Further research should focus on the interaction of stream biogeochemistry with stream draw-down and altered fish communities and the effect this has on stream conditions after re-wetting, given that these

conditions may become more common under current climate change predictions in the Great Plains (Kunkel et al. 2008).

Results for fish effects on GPP were different between path analysis and univariate tests. The path model suggested a direct significant decrease in GPP with the removal of fish and an indirect effect of fish mediated through FBOM (Figure 3.7). The chl *a* → GPP path was not significant in this model, suggesting that fish effects on chl *a* may not carry over to affect GPP rates, which is surprising given the well-documented connection of GPP rates to chl *a* (Vanni et al. 2006, Bernot et al. 2010). The standardized coefficients between Fish and FBOM → GPP suggest that the direct effect of fish was more important than indirect effects mediated through FBOM. Similarly, the unstandardized coefficient for the FBOM → GPP path suggested a minimal decrease in GPP rates (0.01 mg m^{-2} min^{-1}) for every unit increase in FBOM. Direct effects of fish removal decreased GPP rates by 3.92 mg m^{-2} min^{-1}, a much more substantial effect than indirect effects from FBOM.

The lack of fish effects detected for either chl *a* or GPP using univariate analyses brings into question the results of the path analysis model. GPP rates were noticeably higher in 2014 compared to 2013 at both sites (Figure 3.3 A). We corrected for annual differences in the path model using the '*lavaan.survey*' function. This correction only alters the p-value of each path after accounting for the experimental design of the data, while the standardized and unstandardized coefficients remain the same. High GPP rates in 2014 coincided with fish presence, since fish were located at all sites and there wasn't an experimental removal of fish in that year (Table 3.1). Despite our attempt to correct for annual variation, it's possible that direct effects of fish on GPP identified using path analysis may be confounded by similar annual trends at each site.

Abiotic effects on biogeochemical rates

The path from canopy cover → rate was not significant in any of the three models (Figures 3.5-3.7). We predicted that higher canopy covers would result in lower GPP and ER rates due to lower light availability. The lack of a significant relationship between these variables is likely affected by our calculation method of ER and GPP rates. These measurements were taken at different parts of the day at varying light and temperature conditions. Therefore, it was necessary to correct temperature and light conditions to a standard unit (i.e. 20ºC or 300 PAR). In doing so, we may have negated any effect that canopy cover (and light availability) might have had on these rates. Future studies could avoid this by taking measurements at standardized light by covering chambers with a screen or other material that would control light intensity. Alternatively, a previous study on KPBS suggests that NPP doesn't change until canopy cover is greater than 70% (Dodds et al. 1996). Most transects (approximately 75%) measured in this study had a canopy cover below 80%, suggesting that our measurements were taken from transects where we may not expect to see effects on metabolic rates.

The path from D_{50}→rate was significant for all models, while the direction of the effect matched our prediction only for ER (Figures 3.5-3.7). Larger median substrata size typically creates a larger hyporheic zone, and an increase in biogeochemical processing (Findlay 1995). The standardized and unstandardized coefficients were much larger for the D_{50} → ammonium uptake path relative to the ER and GPP paths. Unstandardized coefficients for both metabolism measurements were less than 0.1 (with ER being a negative relationship), while unstandardized coefficient for ammonium uptake was 5.24, suggesting that substrata size might be more important for ammonium uptake rates compared to either metabolism rate. Argerich et al. (2011) found higher ammonium uptake rates (measured with ^{15}N) in cobble compared to sand and mud experimentally manipulated substrata packs.

It is possible that the median substrata size classifications may not be representative of measurements from substrata containers. We filled all containers with homogenized substrata from a dry gravel bed adjacent to the stream, but filled in the areas around the container within the exclosure with substrata removed from the streambed. The biggest differences likely occurred when the substrata in the container was smaller than the transect substrata. If the substrata in the container was larger than the surrounding transect, fine sediments were likely able to fill in substrata containers providing conditions more representative of that transect.

We included width in all path models as a quantitative and continuous measurement of stream habitat (i.e. riffles and pools). Univariate tests suggested a significant difference in FBOM at E3 and ammonium uptake at E1 between riffle and pool habitats (Table 3.3). The Width→ rate path was significant for both GPP and ER rates, and suggested similar increases (0.58-0.87 mg m^{-2} min^{-1}) in rates for a unit increase in width (Figures 3.5 and 3.7). O'Brien and Dodds (2008) identified significantly higher ammonium uptake rates in riffles compared to pools in Kings Creek. Our study identified higher rates in pools compared to riffles at E1using qualitative habitat classifications (Table 3.3), while effects were not detected using width measurements and path analysis. Width may not have been a good estimate of habitat differences in E1, where variation in width between stream habitats was minimal. Stream velocity at each transect may have been a better continuous representation of stream habitat; however, these measurements were not always available.

Conclusions

The results of this study provide evidence for fish effects (primarily *C. Anomalum* and *P. erythogaster*) on both stream structure and function in a prairie stream network. Fish effects were strongest for ammonium uptake when considering results from path analysis and univariate tests

with experimental fish removal. The use of path analysis allowed us to identify indirect effects of fish on ecosystem rates that were mediated through stream structural measurements, and simultaneously test for direct effects of fish and abiotic drivers of biogeochemical rates. Overall, this study suggests that ammonium uptake rates in this system are driven by both biotic and abiotic factors, while metabolism rates are more likely driven by abiotic factors, with the possibility of indirect effects of fish mediated through stream structural components. Varying climatic conditions between years possibly confounded some results (i.e. fish effects on GPP), but also provided insight to future experiments testing the interactive effects of a changing climate, and fish abundance and diversity on ecosystem processes. Biotic, abiotic, and climatic conditions should all be considered when attempting to predict ecosystem rates at larger scales or under different future scenarios.

TABLES AND FIGURES

Table 3.1 Organization of categorical variables (Fish presence, site, and year) among the patch-scale measurements for path analyses.

	E1		E3		
	2013	2014	2013	2014	Total
With Fish	0	10	8	9	27
Without Fish	14	0	8	0	22
Total	14	10	16	9	49

Table 3.2 Initial and final model fit statistics for each path analysis model. Columns 3-5 represent initial model fit statistics. Columns 6-9 represent model fit statics after dropping paths not important to the model (see text for rational of path removal). df = degrees of freedom.

Model	N	df	Model χ^2 test statistic	*p*	Paths removed	Modified df	Modified model χ^2 test statistic	Modified *p*
ER	49	8	8.497	0.752	Site→Rate Fish→ Rate	10	10.93	0.359
GPP	49	8	8.497	0.752	Site→ Rate	9	5.99	0.740
N-Uptake	49	8	8.497	0.752	Site→ Rate	9	4.36	0.886

Table 3.3 Results of 2-way ANOVA between all rates and standing stocks collected at E3 and E1 in 2013 with fish removal and habitat type. Boldface indicates a significant ($p < 0.05$) effect. Groups with an asterisk are ln+1 transformed. This is a slightly modified version of Table 2.3 in Chapter 2 of this thesis. df = degrees of freedom.

			E1		E3	
Response		df	F	*p*	F	*p*
GPP	Fish	1,12	0.10*	0.758	0.03*	0.863
	Habitat	1,12	0.46	0.512	2.05	0.177
	Fish x Habitat	1,12	0.0053	0.943	0.02	0.895
ER	Fish	1,12	0.10	0.752	0.33	0.578
	Habitat	1,12	2.4	0.152	1.05	0.324
	Fish x Habitat	1,12	0.511	0.491	0.09	0.767
Ammonium Uptake	Fish	1,12	2.57	0.139	4.46	**0.056**
	Habitat	1,12	17.86	**<0.01**	1.36	0.265
	Fish x Habitat	1,12	1.70	0.221	2.88	0.115
FBOM	Fish	1,12	1.049	0.329	2.82	0.118
	Habitat	1,12	0.070	0.797	5.41	**0.038**
	Fish x Habitat	1,12	0.374	0.555	2.46	0.143
Chla	Fish	1,12	0.44	0.524	1.40	0.259
	Habitat	1,12	0.33	0.577	1.77	0.209
	Fish x Habitat	1,12	1.88	0.199	0.547	0.474

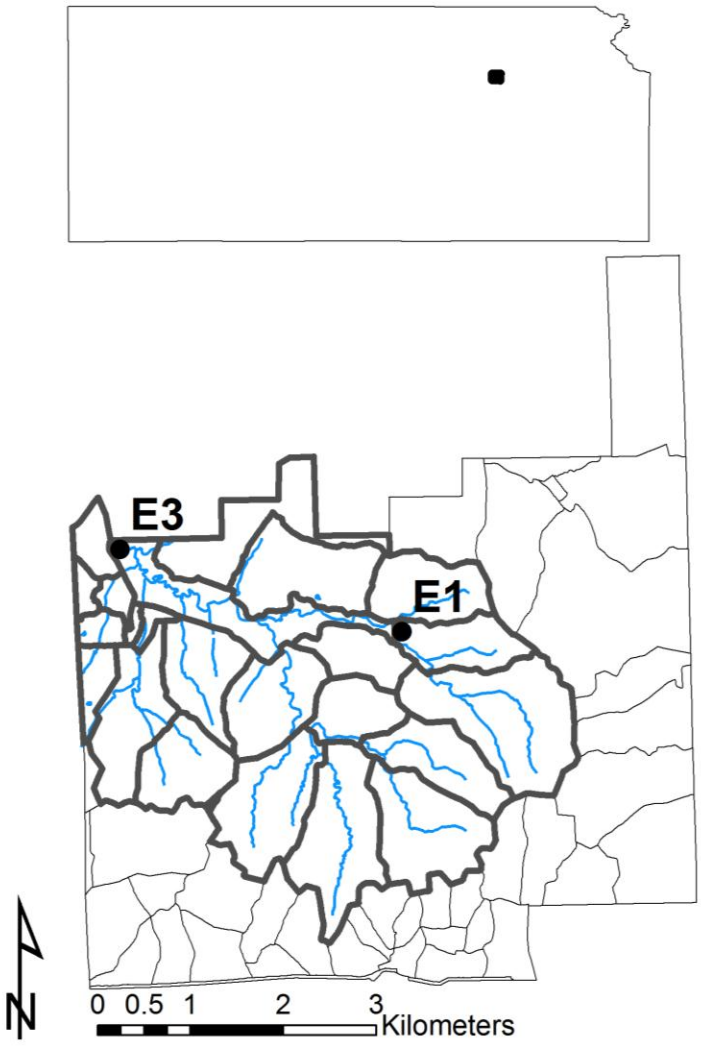

Figure 3.1 Location of sites within the Kings Creek watershed (bold outline) of Konza Prairie Biological Station (light outline).

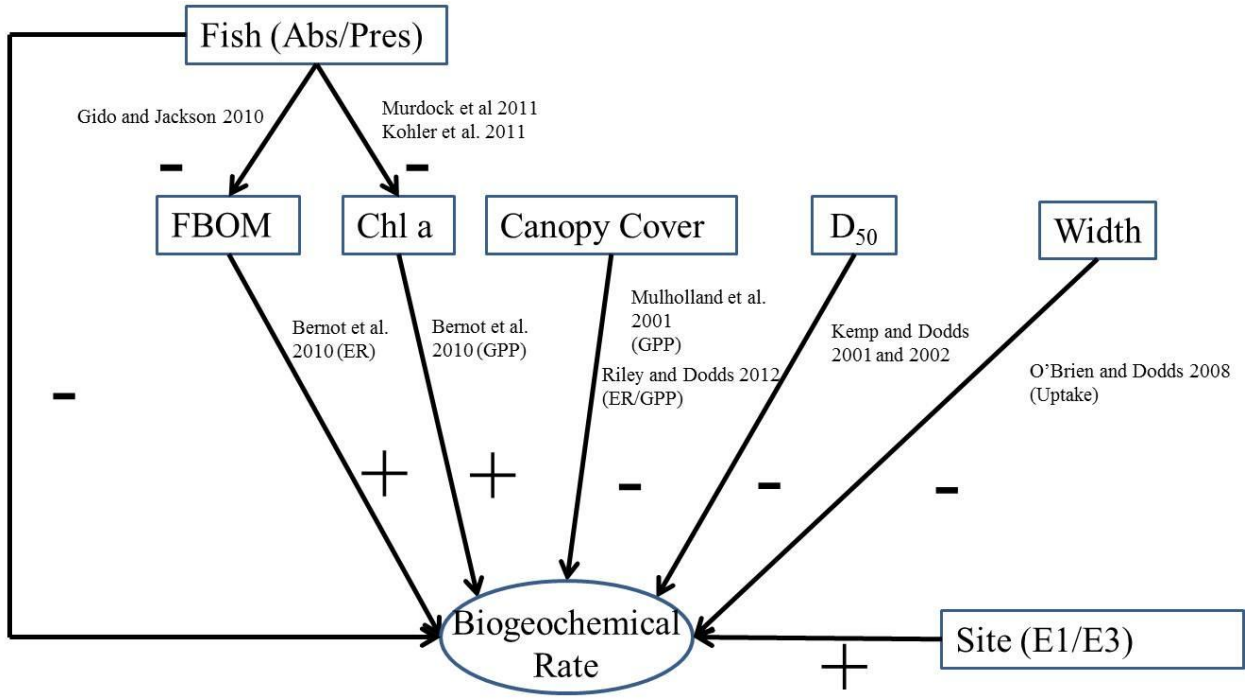

Figure 3.2 Path diagram of expected biotic and abiotic effects for all biogeochemical rates. FBOM= Fine benthic organic matter, Chl a= Chlorophyll *a*, D_{50}= median substrate size (D_{50}), and Width= wetted width of the transect. For processes identified with citations: GPP = Gross Primary Production, ER= Ecosystem Respiration, and Uptake= Ammonium Uptake. Fish and site categorical variables are binary, where 0 represents fish were present and E1, respectively.

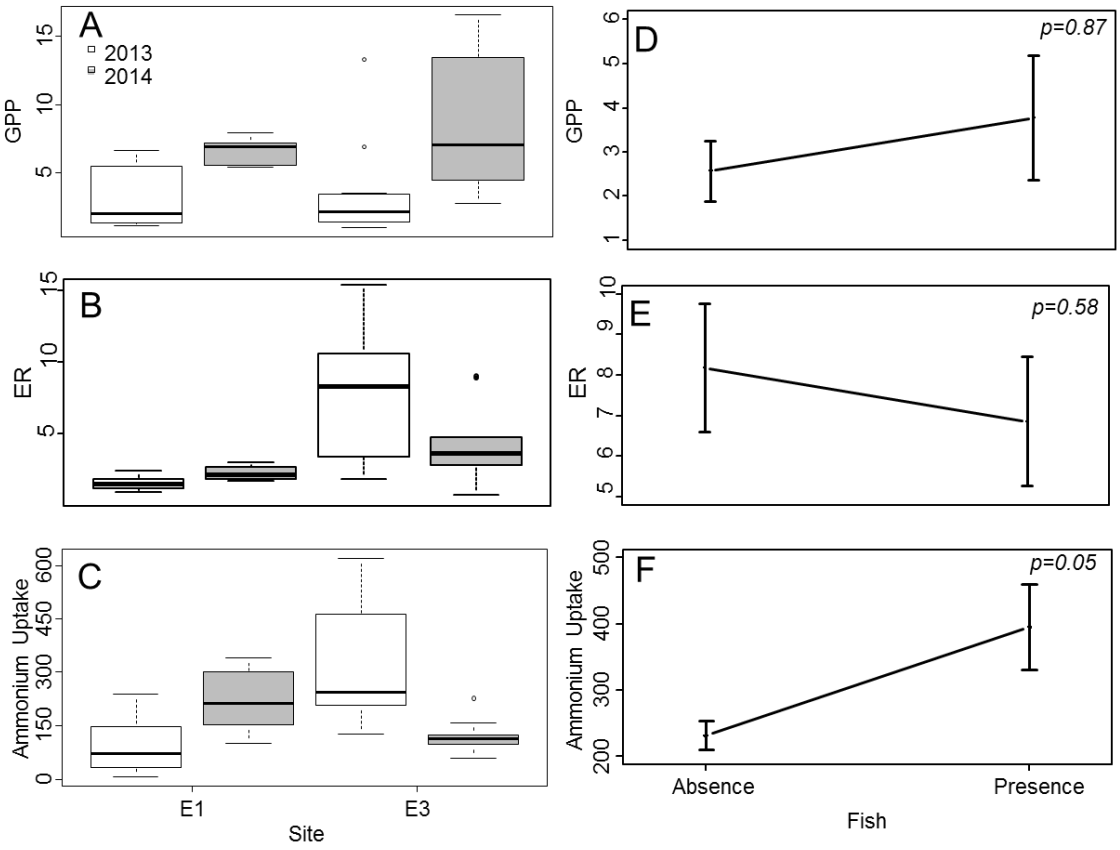

Figure 3.3 Box and whisker plots (A= GPP, B= ER, C=Ammonium uptake) of biogeochemical rates across site and year categorical variables used in path analyses and line graphs (D=GPP, E= ER, F=Ammonium Uptake) of a subset of rates from exclosures at E3 in 2013, where fish biomass was substantially high. Bars within boxes indicated median values. Upper and lower boundaries of boxes represent 25th and 75th quartiles, respectively. Error bars represent 10th and 90th percentiles, and solid circles represent those values outside the 10th and 90th percentiles. Error bars in line graphs represent standard error. P-values represent results of two-way ANOVA between fish removal. Ecosystem Respiration (ER) and Gross Primary Production (GPP) are in units of mg O_2 m^{-2} min^{-1} and ammonium uptake is in units of µg N m^{-2} min^{-1}.

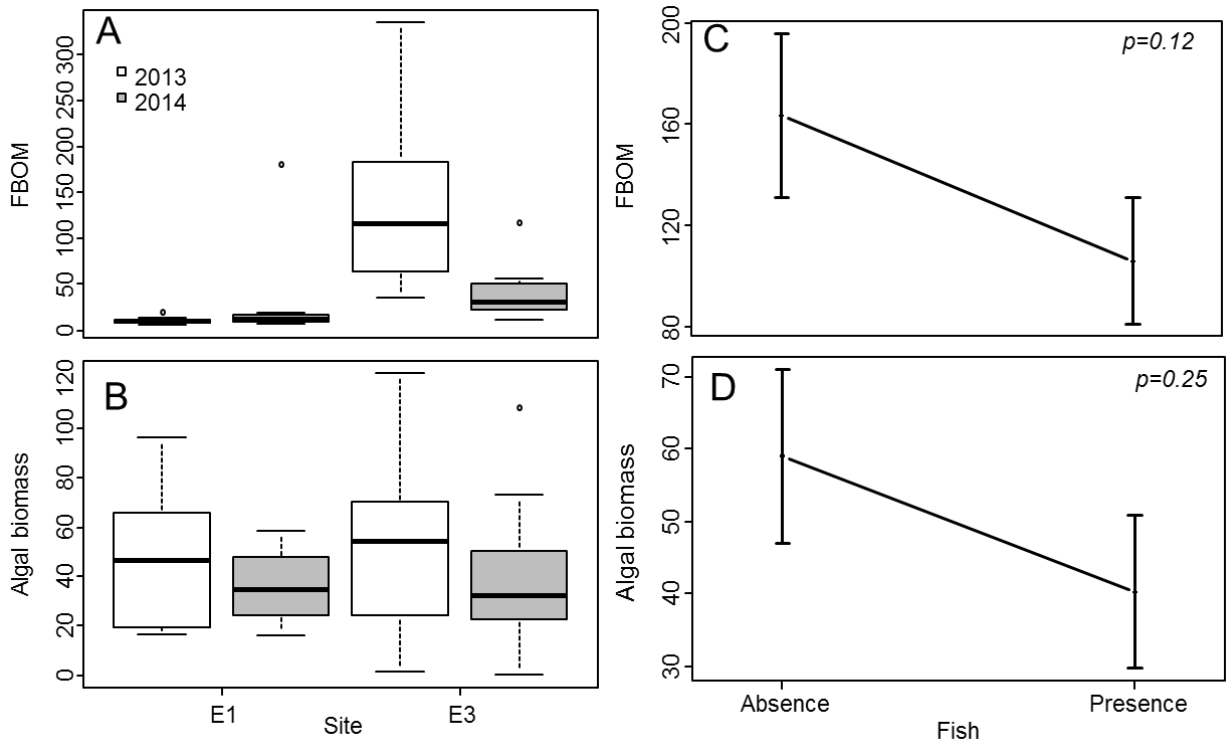

Figure 3.4 Box and whisker plots (A= fine benthic organic matter, B= Algal biomass) of stream structural components across site and year categorical variables used in SEM analyses and line graphs (C= fine benthic organic matter, D=algal biomass) of a subset of rates from exclosures at E3 in 2013, where fish biomass was substantially high. Bars within boxes indicated median values. Upper and lower boundaries of boxes represent 25th and 75th quartiles, respectively. Error bars represent 10th and 90th percentiles, and solid circles represent those values outside the 10th and 90th percentiles. Error bars in line graphs represent standard error. P-values represent results of two-way ANOVA between fish removal and stream habitat. Fine Benthic Organic Matter (FBOM) is in units of g AFDM m^{-2} and algal biomass values are in units of mg chlorophyll *a* m^{-2}.

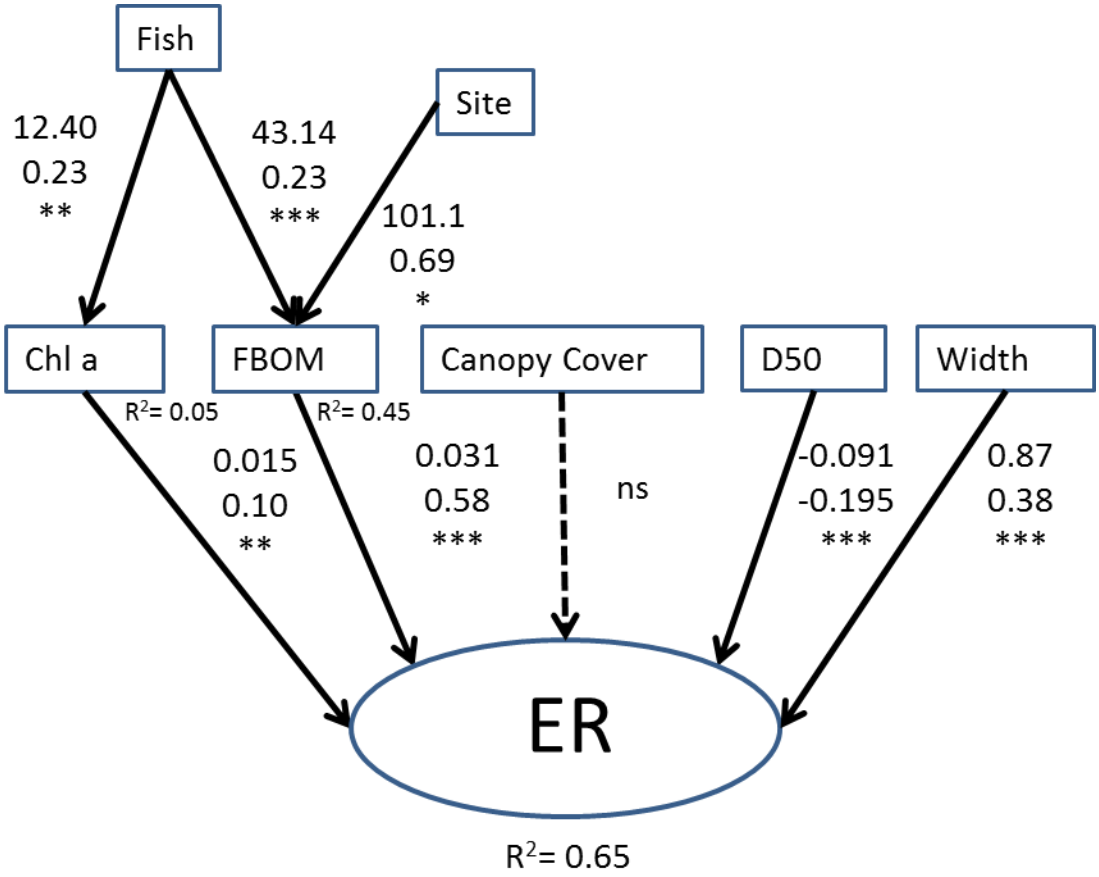

Figure 3.5 Final path analysis model for ER. The numbers associated with each path represent the unstandardized (top) and standardized (bottom) coefficients. The number of asterisks represent the level of statistical significance of the path where: *≤0.1, ** ≤ 0.05, * ≤ 0.01, and ns=not significant. Fish and site categorical variables are binary, where 0 represents fish were present and E1, respectively. Refer to Figure 3.2 for explanation of abbreviations.**

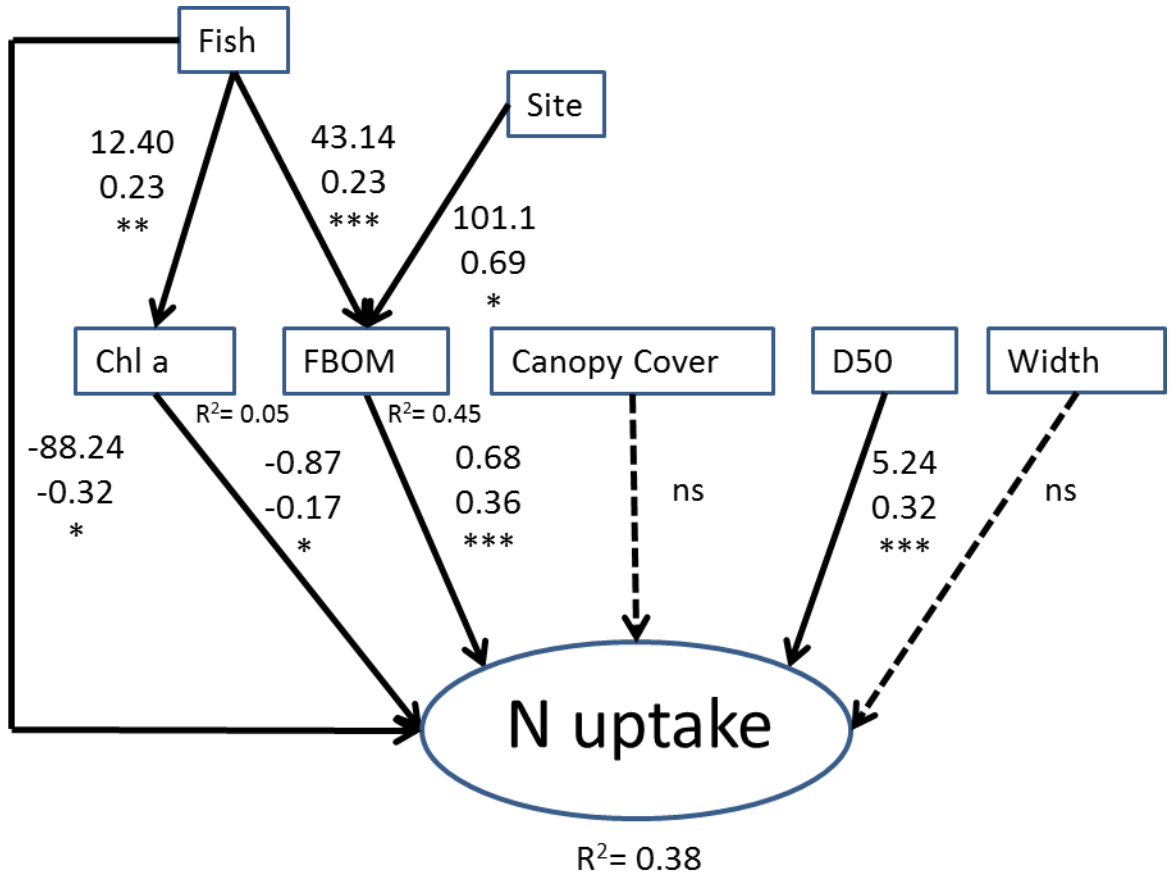

Figure 3.6 Final path analysis model for ammonium (N) uptake. The numbers associated with each path represent the unstandardized (top) and standardized (bottom) coefficients. The number of asterisks represent the level of statistical significance of the path where: *≤0.1, ** ≤ 0.05, * ≤ 0.01, and ns=not significant. Fish and site categorical variables are binary, where 0 represents fish were present and E1, respectively. Refer to Figure 3.2 for explanation of abbreviations.**

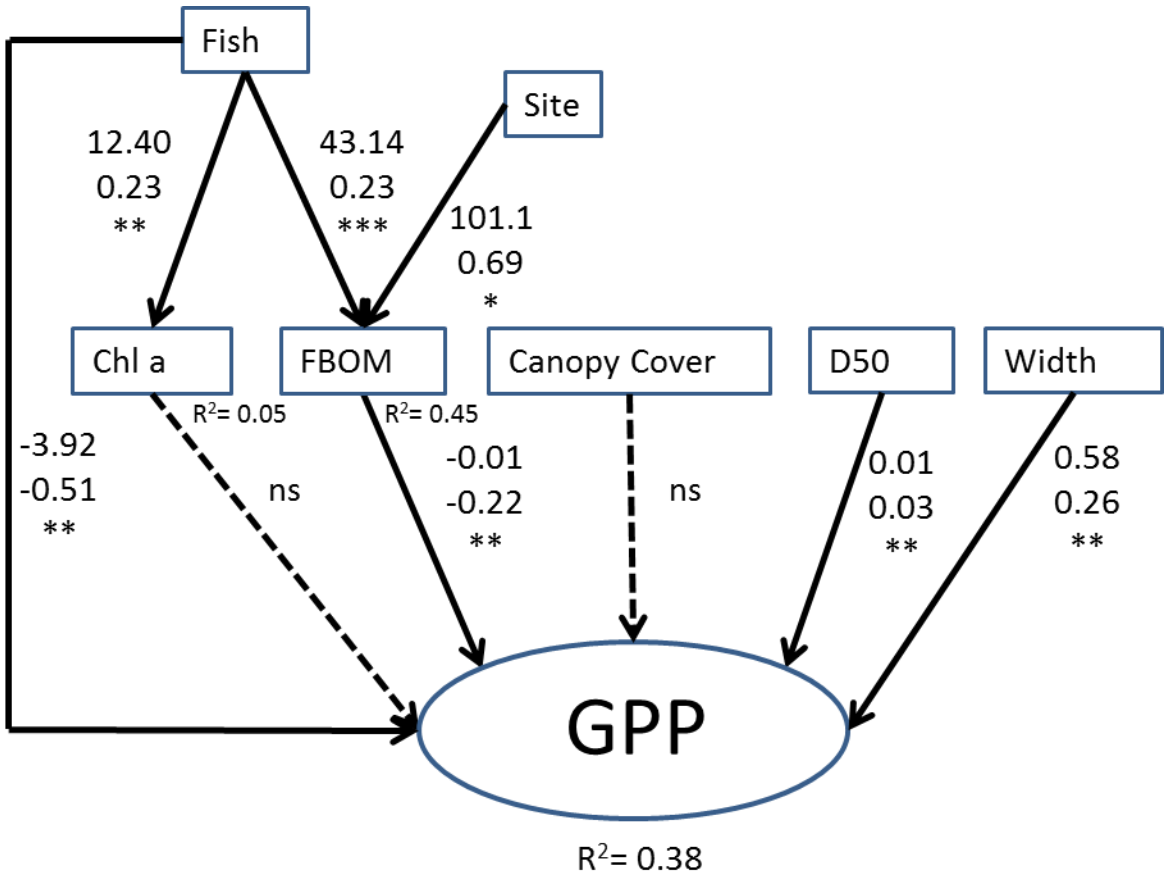

Figure 3.7 Final path analysis model for GPP. The numbers associated with each path represent the unstandardized (top) and standardized (bottom) coefficients. The number of asterisks represent the level of statistical significance of the path where*≤0.1, ** ≤ 0.05, * ≤ 0.01, and ns=not significant. Fish and site categorical variables are binary, where 0 represents fish were present and E1, respectively. Refer to Figure 3.2 for explanation of abbreviations.**

Chapter 4 - Summary and Conclusions

Up-scaling measurements of aquatic processes is important for determining the effects of current and future anthropogenic disturbances at spatial scales relevant to researchers and land managers, as well as for understanding how natural systems function. Headwater streams are a dominant interface between terrestrial and aquatic habitats and process large amounts of nutrients and organic matter before they can reach downstream waters. These streams can exhibit an exceptional amount of abiotic spatial and temporal variability. Thus, it is vital to develop accurate methods for up-scaling aquatic biogeochemical fluxes in these variable systems. This thesis used a simple additive approach to scale nested measurements of biogeochemical rates in a headwater prairie stream, while simultaneously characterizing a variety of biotic and abiotic drivers of these processes.

The first chapter explored the comparability of measured benthic rates of ecosystem respiration (ER) and gross primary production (GPP) at patch (~300 cm^2) and reach (~100 m^2) scales. Observed measurements of abiotic conditions (velocity, substrata size, canopy cover, and course benthic organic matter) coincided with the experimental removal of fish in riffle and pool habitats to test the effect of these factors on patch-scale biogeochemical rates. Patch-scale rates overestimated reach-scale rates for both GPP and ER using additive scaling, after accounting for corrections of temperature and light conditions at the time of measurement, and metabolism rates from a variety of benthic compartments (i.e. macrophyte beds and leaf packs). Fish removal did not alter metabolic rates at patch-scales, suggesting that fish presence may not be an important factor for scaling metabolic rates. Half of the measured abiotic factors differed between stream habitats, highlighting the importance of characterizing these compartments when attempting to predict rates at larger scales. Results from this study suggest that the removal of fish may not

alter benthic metabolic rates at small scales, and more complex models that account for abiotic drivers may be necessary for accurate up-scaling.

The second chapter directly tested the effect of predicted patch-scale biotic and abiotic drivers on biogeochemical rates (ER, GPP, and ammonium uptake) with path analyses and data from the previous chapter. Abiotic factors considered in these analyses include: standing stocks of algal biomass and fine benthic organic matter (FBOM), canopy cover, substrata size, stream wetted width, and watershed location. The effect of fish presence or absence on biogeochemical rates was tested directly and indirectly (as mediated through algal biomass and FBOM). Location of measurements in the watershed was not a significant factor for any of the biogeochemical rates; however, the model suggested location strongly influenced FBOM standing stocks. Fish presence directly increased ammonium uptake, while all rates were indirectly affected by fish through changes in either FBOM and /or algal biomass. Significant paths of abiotic factors varied with each model; however, substrata size was important for all rates. Univariate analyses of a subset of data and path models both agree that biotic and abiotic factors interact to affect ammonium uptake, while GPP and ER rates are likely driven primarily by abiotic factors.

The two studies presented in this thesis showed that (1) there is strong evidence that the presence of fish directly alters ammonium uptake, but not metabolic rates at small scales in prairie streams, and (2) additive scaling of metabolic rates was not sufficient to predict rates at larger spatial scales. In this study, I considered only fish when describing 'biotic' factors; removal of other stream organisms along with fish might result in interactive animal effects. For example, crayfish and freshwater mussels can play an important role on conditions important to biogeochemical fluxes through bioturbation and nutrient excretion (M Trentman unpublished

data). It's unclear how the entire biotic community might interactively alter stream biogeochemical fluxes.

The inability to scale is likely affected by different methodological approaches since reach scale measurements were in situ, while patch-scale measurements required removal of incubated substrata from the stream. Future attempts at scaling should include *in situ* measurements when possible. Spatially explicit scaling approaches (i.e. mechanistic or process based modeling) may be more effective than the additive scaling method used here; however, this study provides valuable insight to the abiotic and biotic conditions that should be considered when building these models. Future attempts to scale biogeochemical rates across all biomes will need to account for the habitat specific variability of abiotic factors and the potential biotic effects of relevant animals, which can be determined through observational or experimental measurements. Finally, climate (flood and drought) played a major role in dictating abiotic conditions during this study, and should also be considered in models predicting future stream biogeochemical rates at any scale.

Overall, this thesis provides a framework for scaling biogeochemical fluxes in any system by accounting for local biotic and abiotic drivers when predicting stream processes at larger spatial scales. I used stream habitat as a means to weight patch-scale fluxes in order to account for differences in biotic and abiotic factors, but other indicators of stream structure could be used to account for spatial variability of a stream. While fish effects on benthic metabolism rates were not detected in this study, the possibility of fish effects is still possible under context-dependent situations (i.e. recovery from flood, stream drawn-down, etc.), and should still be considered given the possible changes in stream characteristics under a changing climate.

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Appendix A - Photosynthesis-Irradiance Curve

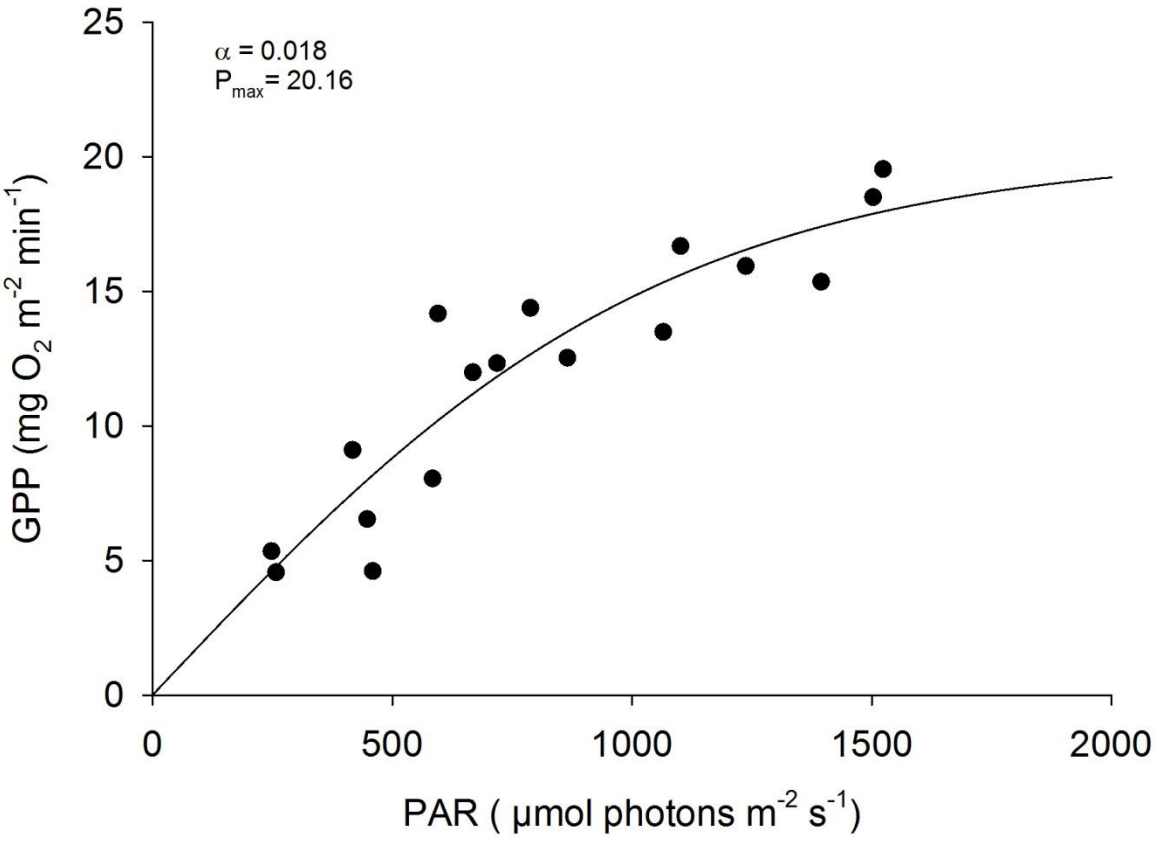

Figure A.1 Photosynthesis-Irradiance curve collected from stream benthic silt/gravel substrata (n=3 replicates) at the patch-scale (300 cm^2). Photosynthesis (GPP) was measured in re-circulating chambers using similar methods as patch-scale metabolism samples from above. The same triplicate set of samples were subject to varying treatments of light using increasing layers of hardware mesh at each light level. The variables Pmax and alpha were calculated by modeling the non-linear equation (Jassby and Platt 1976): GPP=Pmax*tanh((alpha*PAR)/Pmax).

Appendix B - Fish Biomass

Table B.1 A list of species and estimates of fish biomass from reaches in 2013. camano= *Campostoma anomalum*, phoery= *Phoxinus erythrogaster*, ethspe= *Etheostom spectabile*, luxcar= *Luxilus cardinalis*, lepcya= *Lepomis cyanellus*, sematr=*Semotilus atromaculatus*, ethnig=*Etheostoma nigrum*, notexi= *Noturus exilis*

Species code	Site	Timing relative to incubation	Fish treatment in reach	Number of fish	Slope	Intercept	Average individual length (mm)	Average individual biomass (g)	Species biomass (g)	Total reach biomass (g)
camano	E1	post	ambient	8	2.878624	-4.74243	61.14	2.51	20.96	
ethspe	E1	post	ambient	10	3.201493	-5.31949	36.71	0.49	4.67	
phoery	E1	post	ambient	5	3.017257	-5.03806	44.80	0.88	4.40	30.0
phoery	E1	post	removal	22	3.017257	-5.03806	39.50	0.60	13.23	
camano	E1	post	removal	2	2.878624	-4.74243	62.50	2.67	5.35	
ethspe	E1	post	removal	11	3.201493	-5.31949	30.81	0.28	3.13	
sematr	E1	post	removal	3	3.012601	-5.00273	62.33	2.54	7.64	29.3
phoery	E1	pre	ambient	7	3.017257	-5.03806	44.29	0.85	5.95	
camano	E1	pre	ambient	7	2.878624	-4.74243	42.50	0.88	5.76	
ethspe	E1	pre	ambient	19	3.201493	-5.31949	30.21	0.26	5.00	16.7
sematr	E1	pre	removal	1	3.012601	-5.00273	46.00	1.02	1.02	
ethspe	E1	pre	removal	14	3.201493	-5.31949	31.46	0.30	4.19	
phoery	E1	pre	removal	20	3.017257	-5.03806	36.85	0.49	9.70	14.9
sematr	E3	post	ambient	17	3.012601	-5.00273	77.00	4.79	81.46	
ethnig	E3	post	ambient	3	2.953785	-4.99774	52.30	1.20	3.59	
luxcar	E3	post	ambient	2	3.012601	-5.00273	54.00	1.65	3.29	
notexi	E3	post	ambient	19	2.770237	-4.53465	55.00	1.93	36.76	
camano	E3	post	ambient	683	2.878624	-4.74243	56.46	2.00	1363.34	
ethspe	E3	post	ambient	97	3.201493	-5.31949	35.06	0.42	41.02	

phoery	E3	post	ambient	891	3.017257	-5.03806	42.83	0.77	684.43	2213.9
notexi	E3	post	removal	1	2.770237	-4.53465	87.00	6.89	6.89	
camano	E3	post	removal	27	2.878624	-4.74243	52.88	1.65	44.54	
ethspe	E3	post	removal	64	3.201493	-5.31949	35.84	0.45	29.24	
phoery	E3	post	removal	112	3.017257	-5.03806	42.58	0.75	84.32	
sematr	E3	post	removal	8	3.012601	-5.00273	73.41	4.15	34.64	199.6
lepcya	E3	pre	ambient	2	3.012601	-5.00273	26.00	0.18	0.36	
luxcar	E3	pre	ambient	1	3.012601	-5.00273	56.00	1.84	1.84	
ethnig	E3	pre	ambient	4	2.953785	-4.99774	56.50	1.50	6.02	
camano	E3	pre	ambient	212	2.878624	-4.74243	44.38	1.00	211.52	
ethspe	E3	pre	ambient	228	3.201493	-5.31949	34.78	0.41	93.86	
notexi	E3	pre	ambient	330	2.770237	-4.53465	62.62	2.77	914.09	
sematr	E3	pre	ambient	32	3.012601	-5.00273	63.65	2.70	86.38	
phoery	E3	pre	ambient	1044	3.017257	-5.03806	43.98	0.83	868.40	2182.5
camano	E3	pre	removal	37	2.878624	-4.74243	59.00	2.27	83.76	
phoery	E3	pre	removal	354	3.017257	-5.03806	48.18	1.10	387.28	
sematr	E3	pre	removal	32	3.012601	-5.00273	62.29	2.53	79.81	
ethspe	E3	pre	removal	101	3.201493	-5.31949	38.14	0.55	55.71	
ethnig	E3	pre	removal	2	2.953785	-4.99774	60.00	1.80	3.59	610.2