EFFECTS OF RIPARIAN WOODY VEGETATION ENCROACHMENT ON PRAIRIE STREAM STRUCTURE AND FUNCTION WITH EMPHASIS ON WHOLE-STREAM METABOLISM

by

ALYSSA J. RILEY

B.S., University of Wisconsin – La Crosse, 2005

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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Abstract

Much of the North American tallgrass prairie ecosystem has been converted to cropland or urbanized. One threat to the remaining prairie ecosystems, and the streams within, is woody vegetation encroachment. Stream productivity, measured as metabolism, is a fundamental process comprised of gross primary production (GPP) and (CR) community respiration. Understanding GPP and CR is important because these processes are vital to ecosystem function and can be impacted by a change in canopy cover. First, I investigated improvements in existing methods for estimating whole-stream metabolism as estimated from diel patterns of oxygen (O_2). I compared measured and modeled O_2 and aeration (a physical parameter required for measurement of metabolism) rates to determine if direct measurement of aeration is necessary and the importance of temperature correction of metabolism. Modeling was moderately successful in determining aeration rates, and temperature correction of GPP and CR substantially improved model fits. Second, effects of woody vegetation encroachment on prairie stream function were investigated. Stream metabolism was measured for four years in duplicate reaches with varying canopy cover (closed canopy, naturally open canopy, and vegetation removal reaches). The removal reaches had closed canopy for the first two years and open canopy for the last two years. Canopy cover increased CR rates and had minimal effects on GPP. Third, the same experiment was used to determine the effects of woody vegetation encroachment on prairie stream ecosystem structure and food web interactions. Chlorophyll *a* and filamentous algal biomass were greater in naturally open and vegetation removal reaches, although the effects were stronger on filamentous algal biomass. As canopy cover decreased, the filamentous algal biomass to chlorophyll ratio increased, indicating a shift in algal community structure. Stable

isotope analysis indicated some shift in pathways of nitrogen and carbon flux into the food web related to degree of canopy cover, but overlap in the signature of food sources made distinct food sources difficult to identify. The data indicate that riparian encroachment can influence ecosystem structure and function in prairie streams and restoration to remove woody riparian cover may restore some ecosystem features of naturally open canopy streams.

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Table of Contents

List of Figures

List of Tables

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Chapter 1 - Introduction

Prairie streams and woody vegetation encroachment

Historically, the Great Plains area was covered with over 160 million hectares of North American prairie (Samson and Knopf 1994). Prairie was widespread, but human settlement and expansion converted most of the tallgrass prairie land into agriculture or urban areas. Today, less than 5% of this important prairie ecosystem remains intact (Samson and Knopf 1994).

Extensive stream networks ran throughout the tallgrass prairie, and when prairie land was converted the prairie streams were also impacted. There are few protected prairie streams in existence. Prairie streams are important because they are typically spring-fed headwater streams and they play a role in downstream water quality. Nutrient processing and production that occurs in the headwaters can influence these processes downstream (Dodds and Oakes 2008).

In the past, low order prairie streams were surrounded by native tallgrass prairie vegetation. The open canopy associated with prairie streams allowed for full sunlight availability and little allochthonous input in the form of leaves from riparian vegetation. Now, the remaining prairie streams in the Great Plains are faced with the threat of woody vegetation encroachment. Over time the woody vegetation growth along the riparian corridors of streams has changed the open canopy to closed canopy. A change in canopy cover could impact stream structure and function and could potentially alter food web interactions by altering the available food sources.

Kings Creek is a prairie stream whose watershed is encompassed within a protected area, Konza Prairie Biological Station. The expansion of woody vegetation, especially along stream channels, has been well documented for Konza and the surrounding area (Briggs et al. 2005). In just over 60 years, woody vegetation increased approximately 70% (Briggs et al. 2005). If this

expansion rate continues, prairie streams could become forested streams in a short time. The effect of woody vegetation expansion on prairie streams has not been adequately studied and is important to understand because it can influence stream productivity by altering sunlight and allochthonous carbon input.

Stream metabolism

Gross primary production (GPP) and community respiration (CR) are two processes that contribute to whole-stream metabolism. Stream metabolism is a measure of the amount of carbon flux in a system and indicates the ecosystem productivity. There are several methods for estimating whole-stream metabolism (e.g., laboratory or *in situ* chamber estimates, and one-station or two-station open system method). In addition to variation in metabolism methods, there are also differences among researchers in methods for obtaining an aeration rate. Aeration is the flux between the O_2 concentrations in the water column with O_2 in the atmosphere. As water flows, there is a constant exchange in O_2 between the water and atmosphere to reach equilibrium with the atmosphere (saturation), and the rate of this exchange is needed for an accurate estimate of metabolic rates.

Directly measuring aeration in the field requires a precise sampling technique and specific equipment (e.g., gas chromatograph). If direct measurement is not possible, aeration can be modeled (e.g., Atkinson et al. 2008; Dodds et al. 2008) or calculated from empirical equations derived from measurements of physical stream characteristics (Parker and Gay 1987 lists 19 equations from published data). A comparison of direct measurement to modeling aeration and calculating from empirical equations has not been done for small streams.

Main goals

The second chapter of this dissertation describes evaluation of methods for estimating metabolic rates. First, the minimum reach length required for two-station metabolism in Kings Creek (and streams of similar size) was determined. The minimum length determination was done by testing the difference in precise O_2 measurements between upstream and downstream stations. Second, methods for deriving aeration rates were compared to determine the best method for obtaining an accurate aeration rate. Rates from direct field measurements were compared to rates from a non-linear curve fitting model and rates calculated from 19 empirical equations. The third component for assessing methods for measuring metabolism was to explore the effect of temperature on metabolic rates. Temperature-corrected rates were compared to rates that were not corrected for temperature.

In the third chapter, the effect of woody vegetation encroachment on prairie stream function was studied by measuring metabolism in reaches with closed canopy, naturally open canopy, and canopy removal reaches. This study was conducted for four years with the removal reaches as closed canopy for the first two years. Experimental manipulation of the removal reaches consisted of manual canopy removal, leaving the removal reaches with open canopy for the last two years of the study. This allowed for direct comparison of pre- and post-vegetation removal reaches in addition to the comparison to naturally open and closed canopy reaches. Metabolic rates were estimated 4-5 times throughout each year. Metabolism was measured several times in order to account for temporal variability and for estimates to be more accurate.

In addition to stream metabolism, the same experiment was used to explore the impact of woody vegetation encroachment on stream structure and function. The fourth chapter focuses on wood and leaf standing stocks, filamentous algal biomass, chlorophyll *a*, and stable isotope

measurements from closed canopy, open canopy, and the vegetation removal reaches. The standing stock of dead wood from within the stream, leaves, and filamentous algal biomass were estimated by collecting material from within quadrats of known area. Chlorophyll *a* was measured by submersing rocks from the stream in ethanol to extract chlorophyll and spectrophotometric or fluorometric analyses. Biomass and chlorophyll were measured to detect differences in food sources related to canopy cover. Food sources (filamentous algae, leaves, bryophytes, fine benthic organic matter, and epilithon), invertebrates, fish, and crayfish were sampled for stable isotope analysis. Isotope analysis was conducted to determine if food web interactions differed among reaches with different canopy cover. Together, all results were used to determine the impact of woody vegetation encroachment on prairie stream structure and function.

Chapter 2 - Whole-stream metabolism: strategies for measurement and modeling diel trends of dissolved oxygen

Abstract

Stream metabolism is used to characterize the allochthonous and autochthonous basis of stream food web production. The metabolic rates of respiration and gross primary production are estimated by measuring dissolved oxygen concentration in the stream over time. The change in oxygen (O_2) concentration must be analyzed correctly to assess whole-ecosystem function. Various approaches to measuring and analyzing diel O_2 trends have been used previously, but a detailed comparison of different approaches (e.g., required reach length, method of aeration determination, and use of temperature-corrected metabolic rates) is needed. O_2 was measured upstream and downstream of various reaches in Kings Creek, Kansas, using the Winkler method, and we determined that 20 m was the minimum reach length required to detect a significant change in O_2. We also employed models using two-station diel O_2 data and aeration measurements in various streams around Manhattan, Kansas, to assess the potential for accurately modeling aeration and test the importance of accounting for temperature effects on metabolic rates. O_2 was measured at baseflow, and aeration was measured directly with an inert gas and a tracer dye to account for dilution and measure velocity and discharge. Modeled aeration was significantly correlated with measured values (Kendall tau $p = < 0.001$; regression adjusted $R^2 = 0.70$). Nineteen aeration equations from the literature generally provided poor estimates of measured aeration (6 of 19 equations were significantly correlated). Temperature correction of metabolic rates allowed us to account for increases in nighttime O_2. Temperature corrected metabolic rates fit the data somewhat better than uncorrected approaches and can also facilitate cross-site comparisons of metabolism.

Introduction

Metabolic activity in streams is driven by allochthonous and autochthonous production. Stream metabolism indicates total biotic activity and interacts with water quality via basic ecosystem properties such as nutrient uptake rates, carbon flux into the food web and trophic status (heterotrophic and autotrophic state, Dodds 2007). Whole-system metabolism has been measured in streams by using diel trends in oxygen (O_2) since Odum (1956) introduced the method. Gross primary production (GPP), respiration (R, more accurately community respiration, but for simplicity R is used here) and aeration drive changes in O_2 concentration over time. Metabolic rates are estimated by determining how each factor increases or decreases O_2 over distance or time. Net ecosystem production (NEP) is the sum of GPP and R, and these three properties are the fundamental indicators of carbon gain or loss in an ecosystem.

The length of stream reach required to estimate metabolism is not well defined. Reichert et al. (2009) define the reach length required between sampling stations as 0.4v/k, where v is velocity and k is the aeration coefficient. In our case, we needed this information to assess responses in animal exclusion experiments in riffle-pool segments (Bertrand et al. 2009) and we wanted to determine the minimum reach length directly instead of relying on aeration and velocity estimates. Such information verifying the analysis of Reichert et al. (2009) could be useful for other types of experiments requiring reach-specific metabolism estimates.

Aeration rate needs to be known to make accurate estimates of metabolism rates. Some authors have estimated aeration based on physical properties of the stream channel (see Parker and Gay 1987 for list of 19 empirical equations), some have modeled aeration (e.g., Atkinson et al. 2008; Dodds et al. 2008), and others directly measure aeration by using tracer solute and inert gas additions to streams (e.g., Grant and Skavroneck 1980; Genereux and Hemond 1990;

Wanninkhof et al. 1990). Morse et al. (2007) have also related turbulence with sound level to estimate aeration. Given the difficulty and cost of direct measurement, modeling aeration or using simple equations to estimate rates would be preferable. For this paper we assume that directly measured rates of gas transfer give the best possible information on aeration rates for a defined stream reach. Such information is particularly important for two-station metabolism methods. We are aware of some studies that compare methods for estimating aeration (e.g., Aristegi et al. 2009; Kosinski 1984; Young and Huryn 1999). In addition, modeled and measured aeration have been compared for one river (Dodds et al. 2008). However, we are not aware of stream studies that have compared the rates from aeration modeling (using a non-linear curve fitting method) to those obtained from direct measurement and empirical equations across multiple first to third order streams.

Temperature influences metabolic rates (Ambrose et al. 1988; Gulliver and Stefan 1984; Megard et al. 1984) as well as aeration rates (Bott 2006; Elmore and West 1961). Some calculation methods account for diel variation in temperature and others do not (see discussion). We observed that sometimes O_2 concentrations increased over night as stream temperature decreased in some systems. Respiration decreasing throughout the night, most likely driven by decreasing temperature, is one explanation for the O_2 increases. Lower temperatures during the night would increase the saturation concentration of O_2 but decrease the rate of aeration. Thus, we attempt to parse out these temperature effects and explore the importance of temperature correction of metabolic rates in fitting diel patterns of O_2.

Our study focuses on a two-station model for metabolism using measured aeration values. We investigate the following questions: 1) What is the shortest reach that can be used to estimate metabolism, and does this match the predictions of Reichert et al. (2009)? 2) Is it

possible to model aeration with sufficient accuracy that measured values of aeration are not necessary? 3) What is the difference between modeling using temperature corrected and uncorrected metabolism rate estimates?

Methods

The study reaches used to determine the minimum reach length required for measuring two-station metabolism, and several of the stations we collected data to base our model on, were located in Kings Creek, whose watershed is encompassed within Konza Prairie Biological Station (KPBS). KPBS is located in the northern part of the Flint Hills region near Manhattan, Kansas. Detailed site description has been previously published (Gray et al. 1998; Gray and Dodds 1998). This part of the study was conducted in two different subwatersheds, N04D and AL. Watershed N04D has an open canopy or shrub cover and is continuously grazed by the native American bison (*Bos bison*) and burned every 4 years and AL is located in the lower reaches of Kings Creek in the gallery forest. There is a gradient of increasing nutrients between N04D and AL (Kemp and Dodds 2001).

During July of 2005, water samples were taken at the top and bottom of numerous reaches during mid-day and around midnight from N04D and AL to measure small-scale upstream-downstream changes in O_2. These times were chosen to coincide with expected maximal rates of GPP and R. Both sites contained eight contiguous pool and riffle combinations ranging from 7-77 m in length. These reaches were generally cobble bottomed, and had a slope of around 3-3.5%. The modified azide Winkler method was employed with replication because this approach allows for greater precision and accuracy than typical O_2 electrodes. The standard deviation range of the modified azide Winkler assay is reported as 20 μg L^{-1} for distilled water to 60 μg L^{-1} for wastewater (APHA 1995). Standard procedures use 300 mL BOD bottles. For

logistical purposes, this study used 60 mL BOD bottles, with the amount of reagents added adjusted for the volume difference. Samples were collected using a peristaltic pump, run at low speed to avoid cavitation and degassing. The outlet of the tubing was placed in the bottom of the BOD bottle, and it was allowed to overflow 3 volumes worth before the filling tube was gently removed and the stopper placed on the bottle. Six replicate bottles were filled at each site, and reagents were added. Within 6 hours of sampling, O_2 was measured titrimetrically using the modified azide Winkler titration method according to standard methods (APHA 1995).

A Student's *t* test (two sample assuming unequal variances in Microsoft Excel 2003) was used to determine significant differences between the average O_2 concentration of upstream and downstream stations. The p-values were Bonferroni corrected for the number of tests. We took an approach that considered there is some distance below which there will never be a significant difference in O_2, but at longer distances, there may or may not be a significant difference. This minimum distance should lead to a threshold relationship with highly significant changes being detected only above some minimum length. By using an accurate and precise replicated titrametric technique, we could ensure a good detection rate of significant differences between upstream and downstream O_2 concentrations. A two-dimensional Kolmogorov-Smirnov test is a non-parametric method that determines breakpoints in variance for bivariate data (Garvey et al. 1998). This test was conducted on the p-values to estimate the threshold distance below which significant differences were not detectable. In addition, the velocity and aeration values from these reaches in N04D and AL (8 contiguous pool and riffle reaches) were entered into the 0.4v/k equation from Reichert et al. (2009) to calculate minimum reach length requirements and these results were compared to the value obtained from the Kolmogorov-Smirnov test.

We chose streams that varied widely in nutrient content and biological activity to test our modeling approaches. The streams used to determine the best two-station model for measuring metabolism (the effect of modeled versus measured aeration, and the improvement in fit correcting metabolism for temperature) are described in detail in O'Brien et al. (2007). Briefly, we used a range of streams that varied with respect to nutrient content (0.9-21,000 μg L^{-1} NO_3^--N) and degree of canopy cover (0% to over 70% shaded), and included 6 streams from watersheds in native prairie or with various degrees of urbanization or agriculture. The three streams representing prairie/reference streams were two KPBS watersheds, N04D (bison grazed) and Shane Creek (ungrazed), and Natalie's Creek (lightly cattle grazed, 20 km northwest of KPBS). Ag North and Swine Creek were two streams with a small amount of urban area high in the watersheds, and extensive row crop agriculture in the former and row crop and animal holding facilities above the latter. Ag North and Swine Creek had open canopies and high nitrogen concentrations (35 and 21,000 μg L^{-1} NO_3^--N, respectively). Campus Creek was an urban stream with a tree or shrub canopy cover along most of the experimental reach. These 6 streams were all first and second order streams of similar size and slope (Table 2.1). All measurements were made under baseflow conditions. These 6 streams were a subset of 72 streams used in the Lotic Intersite Nitrogen Experiment II (LINX) and in a broad comparison of stream metabolism measures (Bernot et al. 2010).

Two-station diel O_2 curves were measured once in the 6 streams during May and June throughout the time period of 2003-2005 and multiple times in two Kings Creek watersheds, N04D and K02A (ungrazed and burned every two years) during 2006 and 2007 (Table 2.1). The two-station upstream-downstream method (Marzolf et al. 1994; Young and Huryn 1998) was employed at baseflow, using Yellow Springs Instruments logging data sondes set to record

values every 10 minutes. Sondes were calibrated together at a single stream station in the field immediately before deployment. Sondes were placed completely immersed for 30 minutes to be certain the entire sonde was at the same temperature as the water and all sondes were the temperature as each other, as calibration depends upon temperature of the sensor and sonde enclosure. All sondes were calibrated to air-saturated water and allowed to log for 30 minutes. O_2 readings were checked, and if sondes were not reading the same value, calibration was repeated until all sondes gave the same results (within 3%) before deployment. At the end of deployment sondes were again placed together at one station for 30 minutes. If the sondes did not read the same value post-deployment, then the data were corrected assuming a linear drift in calibration over the period of measurement.

Light values were measured using a Li-Cor LI-1000 datalogger equipped with a PAR sensor. Light measurements were logged every hour for the 2003-2005 sites, and every 10 minutes for the 2006-2007 sites. The light sensor was placed on a level elevated object in an open-canopy area next to the stream in full sunlight to determine daily variation in light availability for primary producers. The model requires relative light intensity over the day, so correcting for canopy cover was not necessary.

This study assumes that physically measuring aeration in the field results in the best estimate of the aeration value. At all streams, aeration was measured under similar discharge conditions and in the same reaches where diel O_2 measurements were done using an inert gas (propane or acetylene) and a conservative tracer dye (rhodamine) or ion (bromide). Subsequent measurements of aeration at a subset of the sites using the inert gas SF_6, gave comparable rates and indicated that microbial consumption of the propane or acetylene were negligible in our systems (data not shown). Tracer dye or ion results correct for dilution of the tracer gas and

allow direct measurements of discharge (by dilution) and travel time (time for half the plateau concentration to be reached at the downstream station). The tracer ion and dye also ensured that plateau was reached downstream before gasses were sampled. Dye and ion solutions were dissolved in reverse osmosis water and released at a continuous rate at the same time as the gas using an FMI lab pump (Fluid Metering, Inc. model QBG). The gas was released into the stream through an airstone. The airstone and the tube releasing the dye or ion were positioned inside a T-shaped PVC tube placed upstream from the first sampling site to ensure that gas and tracer dye or ion were thoroughly mixed before the most upstream sampling point (Dodds et al. 2008).

Rhodamine fluorescence was determined in the field using a handheld Aquafluor fluorometer (Turner Designs model 8000-010) and bromide in the field with a handheld ion-specific probe. Once measurements reached a plateau downstream (no more than 1% change per minute), complete mixing was assumed and sampling for dissolved gasses and dilution of the tracer began. For five of the LINX streams (Ag North, N04D, Campus, Swine, and Shane) gas replicates were measured at varying points along the stream reach. For Natalie and the sites from N04D and K02A for 2006 and 2007, replicates of gas samples were measured at the top and bottom of the reach.

At each gas sampling point, 40 mL of water were slowly drawn (so cavitation did not cause degassing of the solution) into a 60 mL syringe with a 3-way stopcock attached. Each syringe had 20 mL of helium (gas chromatography carrier gas) drawn into the syringe and shaken for 3 minutes to strip the tracer gas out of solution. Then the 20 mL of gas was injected into an evacuated vial (vacutainer, 15 mL). The remaining solution was analyzed for tracer ion or dye concentration to account for dilution on a sample-specific basis. Samples in vials that did not maintain vacuum were discarded. The gas samples were analyzed within 24 hours with a gas

chromatograph (Shimadzu GC-14A) equipped with a flame-ionization detector. The difference in average gas peak area from points along the reach, or upstream to downstream (depending on the site), was used to calculate the aeration coefficient.

Standard error was calculated on the measured gas values depending on how gas samples were collected. For streams where gas was measured longitudinally, standard error was obtained from regression analysis (Microsoft Excel 2007). For streams that only had gas measured at the top and bottom of the reach, a pooled-Student's *t* test was used to test for significant differences from upstream to downstream, and standard error was obtained by calculating the pooled standard error on the difference between the upstream and downstream gas replicates.

Physical measurements of the stream habitat were taken to model metabolism. Length and average width measurements were taken in all reaches to calculate discharge and travel time. Width measurements were made every few meters along the length of the reach and 5 depth measurements were taken across each width transect.

Physical measurements and O_2 trends were used to parameterize a model to estimate aeration and the sensitivity to temperature correction of metabolism rates. The basic modeling approach was to calculate O_2 every 10 minutes as influenced by rates of photosynthesis, respiration, and aeration. The Solver option in Microsoft Excel was used to find the best fit of our modeled O_2 to observed O_2. We used the Solver to minimize the sum of squares of error (SSE) between modeled and measured values by changing the basic rates (photosynthesis, respiration and aeration) that drove the model.

Basic data for every model run were supplied by the diel O_2 and temperature values for each 10 minute time period from the sondes. Additional data required for the model included reach characteristics (length, depth, width, average velocity, and discharge), barometric pressure,

and light. The temperature and O_2 values were offset by the calculated travel time. All of the variables (Table 2.2) and equations (Table 2.3) used in constructing the model are provided. The model spreadsheet is available from the authors upon request.

Aeration was corrected for temperature as in Bott (2006) where the equation was adapted from Elmore and West (1961). Elmore and West (1961) stated that 1.0241 should be used as the temperature coefficient and Bott (2006) used 1.024 for the coefficient number. R was corrected for temperature using a relationship adapted from Parkhill and Gulliver (1999). In cases with no correction for temperature, single values of R_T were used regardless of temperature. GPP was modeled with a hyperbolic tangent model of Jassby and Platt (1976) to link photosynthesis and irradiance. Inhibition is generally not observed in intact periphyton assemblages (Dodds et al. 1999) and was not modeled. In model runs with correction for temperature, we used an equation from Parkhill and Gulliver (1999) to modify P_{max} as a function of temperature. In cases where there was no correction for temperature, single values of P_{max} and α were used.

We ran three general model scenarios. In the first scenario GPP, R, and aeration were corrected for temperature and Solver changed $Pmax_T$, α_T, k, and R_T to minimize the SSE between measured and modeled O_2 values. Then measured versus modeled aeration values were compared to determine the ability to predict aeration with modeling while trying to use the three parameters to fit the observed data. It was assumed that measured aeration was more accurate than modeled aeration, and thus all subsequent model scenarios were run with measured aeration rates. Two scenarios [full temperature correction (temperature corrected GPP, R, and measured aeration) and only measured aeration temperature corrected] were created and p-values from a paired Student's *t* test (paired 2 sample for means in Microsoft Excel 2007) across all measured

sites for each scenario were compared to each other to determine significant differences among the two model scenarios.

Measured aeration values were compared to the modeled values and the aeration calculated from 19 empirical equations using a nonparametric Kendall Tau correlation analysis (STATISTICA 6.0 by StatSoft, Inc.). This determined the correlation between the measured and modeled aeration values. The empirical equation that was significantly correlated with the measured aeration value and had the highest R^2 from regression analysis was deemed the best empirical equation. All empirical equations were corrected at 20^o C as stated by Parker and Gay (1987). Direct measurement was compared to values from modeling and empirical calculation. Variance explained by regression, and significance of slope against an expected slope of one, as well as root mean square error and mean error as estimates of bias were used to compare approaches with data from the 6 LINX streams to determine the best method for obtaining an aeration rate.

Results

Reach length needed to detect a significant difference in O_2 concentration between an upstream point and a downstream point was investigated. The p-values from a Student's *t* test of each O_2 upstream-downstream pair were used to compare the statistical difference between stations (Fig. 2.1). The results were Bonferroni corrected; therefore tests with a p-value > 0.006 should not be considered significant. A two-dimensional Kolmogorov-Smirnov test revealed a breakpoint in the variance at 20 m, and below this length, p-values were generally > 0.006. At lengths greater than 20 m there were significant and insignificant results indicating that at greater distances O_2 may or may not be different between upstream and downstream points, and would be dependent on the productivity in the stream. These data suggest 20 m is the minimum

distance required to detect a difference in O_2 given the metabolic and aeration rates in this stream, and the precision of the O_2 assay. The equation from Reichert et al. (2009) resulted in a median predicted reach length of 25 m across the sites, which is comparable to the 20 m reach length determined in the current study.

The change in O_2 was measured using the Winkler method at mid-day and around midnight across the contiguous reaches to find the maximum periods and locations of effect on O_2 concentration (Fig. 2.2). Our Kings Creek sites (watershed N04D and AL) started with O_2 below saturation in the first pool, and then by the second pool the O_2 increased. For these two watersheds during the night and day, O_2 at the most downstream point was greater than at the most upstream point. At both sites the reaches were fed by low O_2 groundwater at the top of the reach. The groundwater effect is demonstrated by the increase in O_2 downstream as the groundwater effects dissipate as distance increases from the groundwater source. It is also evident that O_2 increases less during the night than during daytime because biological processes should not create O_2 at night. This situation illustrates the importance of using the two-station method over the single station method to measure metabolism when groundwater influences are present. A single station method would assign the O_2 deficit in the stream to respiration, where in reality that respiration could have occurred anywhere in the groundwater or soil water above the reach.

Measured aeration values corrected to 20 ^{o}C measured in the 6 Kansas streams from 2003-2007 (LINX sites and additional reaches on KBPS) were compared to the full temperature corrected modeled aeration values using a regression and Kendall Tau correlation analysis (Fig. 2.3). Aeration was measured at some sites more than one time (N04D and K02A); however values did not correlate from year to year, so we assumed pseudoreplication was not a concern.

Including all 16 data points resulted in a correlation p-value of < 0.001 and an adjusted R^2 value of 0.70, demonstrating that modeling aeration may be a useful, viable approach.

Using regression and Kendall Tau correlation analysis, the measured aeration values for the 6 LINX Kansas streams were compared to modeled aeration values and values from 19 published empirical aeration equations (Table 2.4). The measured aeration values were significantly correlated with 6 equations, five of which had a p-value of 0.039. The equation from Parkhurst and Pomeroy (1972) was most closely correlated with the measured aeration ($p = 0.015$) and this equation incorporated velocity, channel slope, stream depth, and the Froude number. Even though the Parkhurst and Pomeroy (1972) equation had the highest correlation with measured aeration, the regression was a poor fit ($R^2 = 0.172$, data not shown). Of the six equations that were significantly correlated with measured values, the equation from Tsivoglou and Neal (1976) had the highest adjusted R^2 value (Fig. 2.4). Tsivoglou and Neal (1976) incorporated travel time and the difference in elevation from the top to bottom of the reach into their equation. The adjusted R^2 values were 0.59 and 0.72 respectively for modeled and calculated aeration regressed against measured aeration for these 6 sites, respectively. However, the predicted slope of the relationship between modeled and measured aeration was not significantly different from one ($p > 0.05$) whereas the slope of the calculated versus measured aeration was significantly less than 1 ($p < 0.05$). Root mean square error was 1.8 times greater for the comparison using calculated rather than the modeled aeration. Finally, while both modeled and calculated aeration underestimated measured aeration (the mean error was negative) the mean error was 3.2 times greater for measured versus calculated as opposed to measured versus modeled aeration. This analysis suggests greater bias is introduced when using calculated as opposed to modeled values to estimate measured aeration.

The R, GPP, and SSE results for model scenarios (using the measured aeration value) of full temperature corrected and only aeration temperature corrected were evaluated to determine which model scenario would be the most accurate at estimating metabolism. A Student's *t* test (paired 2 sample for means) was used for a comparison of R from both model scenarios and resulted in a p-value of 0.02. The same test was done to compare GPP for both model scenarios and resulted in a p-value of 0.03. After Bonferroni correction for two tests ((0.05/2)=0.025), R was significantly different between the two model scenarios, and GPP was marginally significantly different between the two model scenarios. Therefore, temperature correction gave significantly different estimates for daily R and GPP. When comparing SSE results for the two model scenarios, four of the six cases resulted in a lower SSE in the full temperature corrected scenario. Since it is widely accepted that temperature does affect aeration, and most researchers account for this, we decided the best scenario was the full temperature corrected model because in 4 of the 6 cases the SSE was lower (by 0.2-14.7) when GPP and R were also temperature corrected. Using the full temperature corrected scenario also allows us to explain the observed nighttime increase in O_2, although this did not strongly influence overall SSE.

When only aeration was temperature corrected and R and GPP were not, then modeled daily R rates were estimated to be on average 10% lower than in the full temperature corrected scenario. Shane had the smallest difference in R between the two model scenarios, with R being 3% lower when it was not corrected for temperature than when it was corrected. N04D had the biggest difference in R with the rate being 18% lower when it was not corrected for temperature. For GPP, the range of difference between the two model scenarios was 1% lower for Campus to 50% lower for Natalie when GPP was not corrected for temperature. The average difference in

GPP rates was 14% lower when it was not corrected for temperature than in the full temperature corrected scenario.

Daily NEP rates for the 6 Kansas streams from both model scenarios indicated that 3 streams were net heterotrophic and 3 streams were net autotrophic (Table 2.5). Although the heterotrophic state of the streams did not change between the two model scenarios, the magnitude of the metabolic rates was different. For NEP, the difference in rate estimates ranged from 4% greater to 300% lower in the only aeration temperature corrected scenario than in the full temperature corrected scenario.

Using the Ag North stream as an example, O_2 change driven by R, GPP, and aeration were compared for both model scenarios (Fig. 2.5). O_2 change from R decreased during the nighttime and varied from -2.7 to the daytime maximum of -4.0 (mg L^{-1} reach $length^{-1}$) for the full temperature corrected scenario. The aeration only temperature corrected scenario resulted in a constant R value of -4.7 (mg L^{-1} reach $length^{-1}$) throughout the day. The full temperature corrected scenario resulted in an O_2 change from GPP that ranged from 0-9.4 (mg L^{-1} reach $length^{-1}$). Correcting only aeration for temperature led to a greater swing in GPP values with a range of 0-12.0 (mg L^{-1} reach $length^{-1}$), to offset the fact that the R value for the temperature corrected model had a mean greater than when it was not temperature corrected. O_2 change from aeration showed similar patterns to O_2 change from GPP; with aeration temperature corrected being the most variable with a range of -7.5 to 4.5 (mg L^{-1} reach $length^{-1}$). The full temperature corrected scenario had a range of -4.0 to 2.4 (mg L^{-1} reach $length^{-1}$). Both model scenarios reached the maximum O_2 change from aeration during the nighttime when temperatures were the lowest. Comparing the measured and modeled values of change in O_2 for Ag North demonstrates how well the modeled values can fit the measured values (Fig. 2.6).

The Ag North example does not exhibit the increase in O_2 during the night, as was observed in some cases. For example, Natalie's Creek did have an increase in nighttime O_2. Comparing the measured and model values of change in O_2 for Natalie's Creek still shows a good fit between the two sets of values (Fig. 2.7). Downstream measurements of O_2 and temperature from Natalie's Creek show O_2 increasing during nighttime as temperature decreases at night (Fig. 2.8). The full temperature corrected model allows for an explanation of the increase in nighttime O_2. Aeration and R both decrease during the night (Fig. 2.8). Overall aeration rate decreases at night because R is decreasing and not forcing the system as far from saturation.

Discussion

Whole-stream metabolism has been measured in various ecosystems and in reaches of varying lengths. In a broad study of nitrogen metabolism, 72 streams were characterized, and discharge ranged from 0.01 to 16.08 m^3 min^{-1} (Mulholland et al. 2008). The discharge of our streams was within this range. The slope of our streams also fell within the range of slopes for the 72 streams (Bernot et al. 2010). These data suggest that the streams used in this study are not atypical of other streams where metabolism has been measured.

Researchers commonly use either the one-station or the two-station method for calculating whole-stream metabolism. The one-station method consists of O_2 measurements from one point in the stream and the two-station method uses an upstream point and a downstream point. This study focused on the two-station method because this method allows for measuring metabolism in a physically defined reach. In systems where there is a strong upstream influence on O_2 concentration, for example groundwater input, it is strongly suggested that the two-station metabolism method be used. For example, if there was low O_2 groundwater

entering the stream and the one-station method was used, metabolism calculations may indicate a greater R rate than was actually occurring in the stream.

When using the two-station method it is beneficial to know the minimum reach length required for measuring significant differences in O_2. Our results suggest that it is likely that a reach less than 20 m in length cannot be used to assess whole-stream metabolism for streams of similar physical, hydrological, and biological characteristics. Our data matched the predictions of Reichert et al. (2009). This information is valuable for experimental design and application, although it should be viewed as only a rough guide for other streams, and most useful for streams similar to those used in this study.

Aeration can be measured, modeled, or calculated empirically from literature equations and calculation methods (i.e. energy dissipation, surface exchange, nighttime regression). The nighttime regression method (Hornberger and Kelly 1975), which is a common method, was not used here because nighttime regression assumes that nighttime R is constant. This was not a reasonable assumption for several of the reaches during this study.

Physically measuring aeration in the field is technically difficult. It would be simpler and more cost effective to model or calculate aeration, and this study has demonstrated that modeling aeration could be a viable method for determining this value as required to estimate whole-stream metabolism. Direct measurement of aeration in the specific reach used for two-station metabolism is the best option for obtaining aeration estimates, as it directly measures gas flux rates between the two defined points in the stream. Modeling aeration in conjunction with R and GPP could be less accurate because the three parameters can co-vary and give similar results. For example, if R and GPP rates are doubled, then doubling aeration rate will lead to similar diel patterns of O_2. If measuring aeration is not possible, the next best way to obtain an aeration rate

would be to model it using a non-linear curve fitting method. The least reliable option for obtaining an aeration rate for streams similar to ours would be from empirical equations.

Based on correlation and regression analysis, the aeration equation from Tsivoglou and Neal (1976) would be the best of the 19 empirical aeration equations compared for streams of similar physical and hydrological characteristics. Even though the measured values were below the 1:1 line, indicating that the measured aeration values are greater than the values calculated from the Tsivoglou and Neal (1976) equation, the regression is a strong fit. Low correlation across most empirical equations as a function of measured aeration indicates that measuring or modeling aeration would be more accurate than using literature equations.

Temperature correcting aeration is extremely important because the rate of aeration increases by 2-4% per 1 ^{o}C change for a temperature range of 5 ^{o}C to 30 ^{o}C (Owens 1974). In streams such as ours, the maximum diel temperature variation was about 9 ^{o}C, which could lead to an 18-36% variation in aeration rates across a diel period.

Error in modeling or measuring aeration can contribute to error in determination of metabolic rates, but it is not the only source of error in calculating metabolic rates. More detailed discussion on additional sources of error (such as measurement of travel time and instrument calibration) can be found in McCutchan et al. (1998). Methods for calculating or modeling metabolism also vary among researchers, and this is another potential source of difference among studies. For example, we used an Arrhenius coefficient of 1.045 to account for the effect of temperature on R (Ambrose et al. 1988; Gulliver and Stefan 1984), whereas Naegeli and Uehlinger (1997) used an Arrhenius coefficient of 1.07. This temperature coefficient could differ, and we have no way to know which one of these is correct for whole-system metabolism. The theory of temperature corrections of biological rates is still debated (del Rio 2008), and it is

not known how the metabolism summed across entire ecosystems responds to temperature in streams found in different biomes.

The modeling method using non-linear curve fitting approaches is somewhat subjective. Iterative numerical methods such as those used by Excel's Solver can find locally stable solutions that are not globally optimal. Thus, we recommend inspecting graphs of measured versus modeled values and comparing to determine if the fit is good and the SSE is minimized. We took this approach, and when fitted curves did not match data, the first step was to re-run the model with altered initial parameters. If this did not correct the mismatch of data, or generation of nonsense results, the original O_2 data were re-examined for anomalies in our O_2 data that thwart modeling efforts. For example, animals occasionally would enter sonde housings causing a drastic short term dip in O_2 (perhaps the case between 2000 and 2500 minutes in figure 2.7). In obvious cases, the diel O_2 trace was corrected. In some cases the entire run needed to be discarded due to general sonde malfunction.

Temperature correction is important not only because it accounts for the observation that O_2 increases at night in some streams, but also because it allows for cross-site comparison of metabolism values. Respiration rates can change overnight (Jones et al. 1995; Tobias et al. 2007). Some studies correct R for temperature (e.g., Parkhill and Gulliver 1999), and some studies do not correct R for temperature (e.g., Bernot et al. 2010; Bott 2006; Marzolf et al. 1994; Mulholland et al. 2001).

The model presented here accounts for the major factors that could influence metabolism over periods of days in streams under stable flow conditions or no unusual disturbances. The model corrects GPP for light and temperature, whereas many other calculation approaches do not account for light explicitly.

Conclusions

Knowing that a reach of at least 20 m is needed to detect a significant difference in O_2 is useful information that will aid in the design of future experiments. This is comparable to the median of 25 m determined from the equation in Reichert et al. (2009). The benefit of determining reach length as reported in the current study is that an aeration estimate was not required. In the extreme case, aeration can be too high for any metabolic measurement regardless of reach length. If aeration was very low, and water replacement (mean velocity) low, substantially shorter reaches might have yielded significant results.

Measuring aeration minimizes the possible error that comes with modeling aeration, and probably ultimately yields the most accurate estimates of metabolic rates. However, a non-linear curve fitting model could provide reasonable aeration estimates if directly measuring aeration is not possible. Temperature can influence R, GPP, and aeration so correcting these parameters for temperature as suggested in this study and incorporating light influences on GPP into the model allows for stronger cross-site comparisons of metabolism, and a closer fit between observed and modeled O_2 dynamics in streams.

Figure 2.1 Statistical differences between O_2 stations using a Student's *t* test (two sample assuming unequal variances). Based on Bonferroni correction, results with a p-value above 0.006 (horizontal dashed line) were not significant given the number of tests. A two-dimensional Kolmogorov-Smirnov test suggested 20 m as the breakpoint in the relationship (vertical dashed line). We obtained no statistically significant differences with less than 20 m reaches, suggesting a 20 m long reach is needed to measure significant differences in O_2 given the metabolic and aeration rates in this stream.

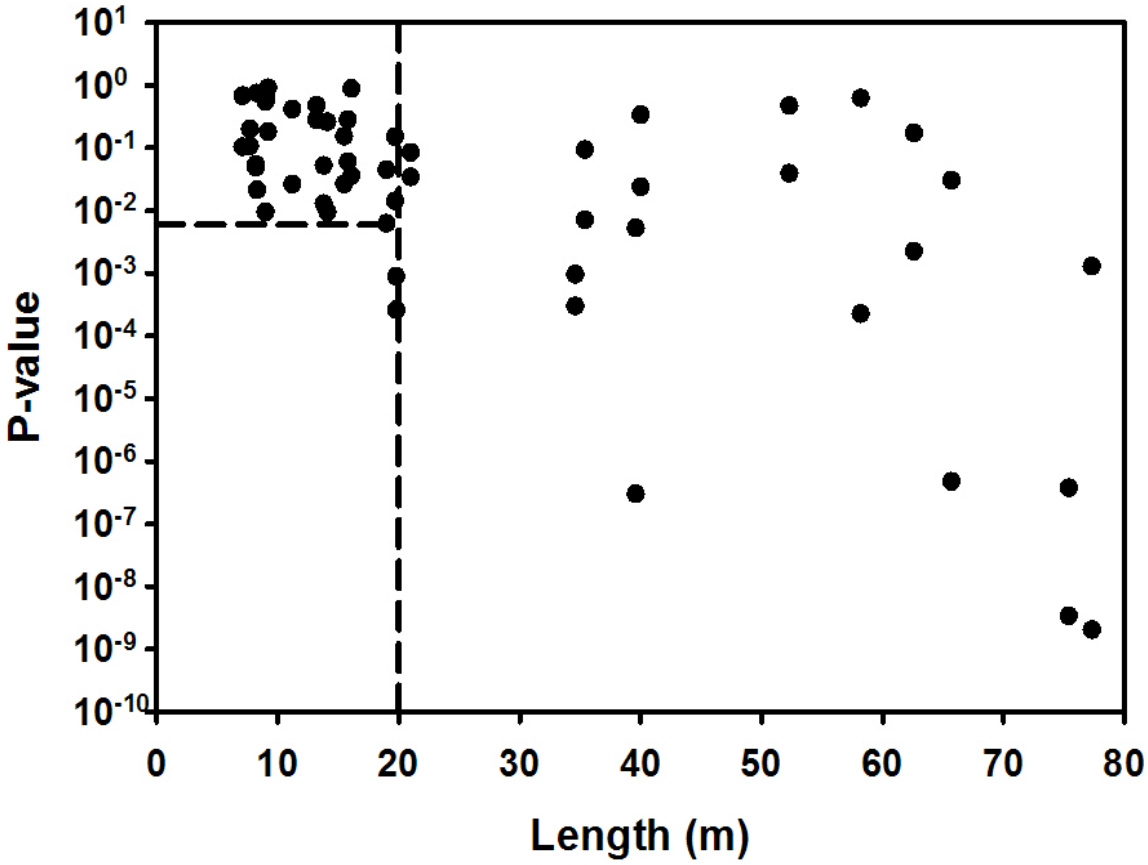

Figure 2.2 Winkler O_2 measurement versus distance downstream during the night and day from two different subwatersheds. Measurements were taken during July 11-19, 2005. Error bars represent $\pm$ 1 standard deviation on the Winkler O_2 measurements (range of 0-7.6). In both cases the reaches were fed by low O_2 groundwater at the top of the reach.

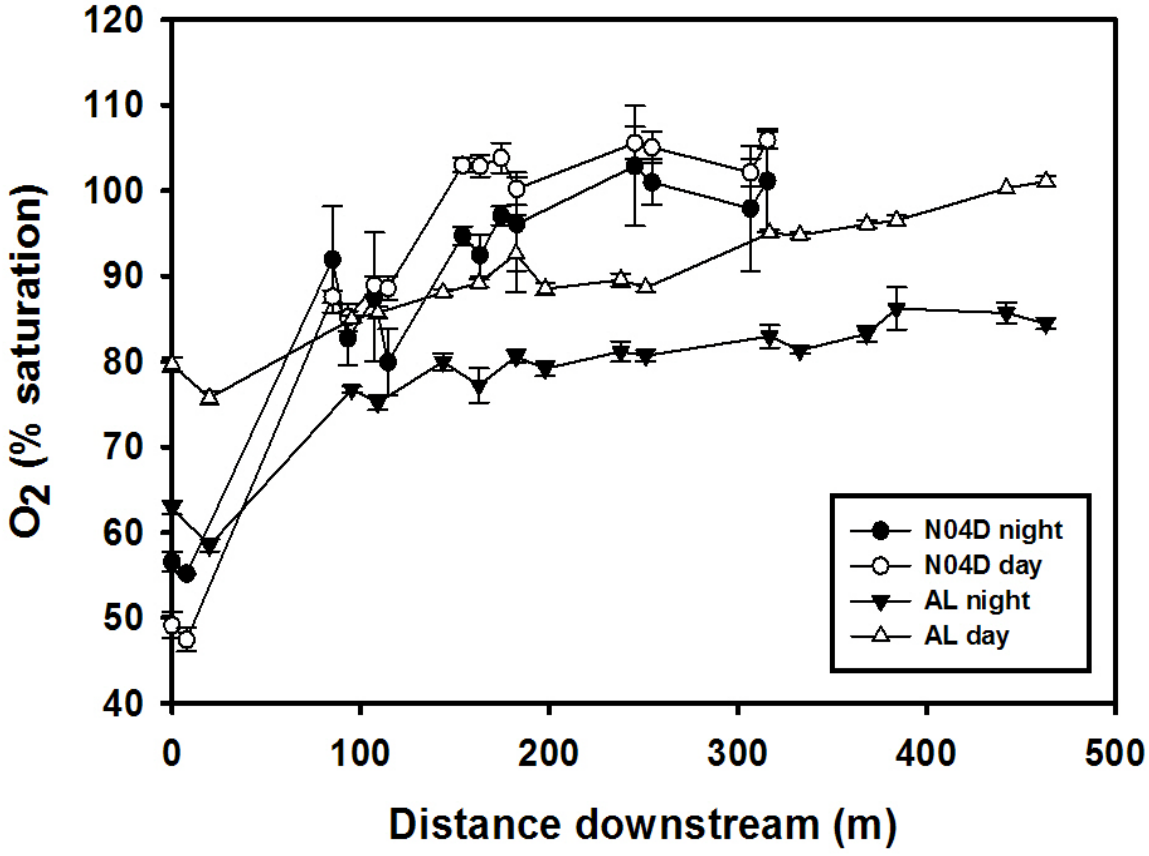

Figure 2.3 Correlation (Kendall Tau, p = < 0.001) of measured and modeled aeration values corrected at 20 ^{o}C from all sites. Regression analysis resulted in an adjusted R^2 value of 0.70 and an equation of y = 0.9505x – 0.0021. Error bars represent standard error and the dashed line represents a 1:1 line.

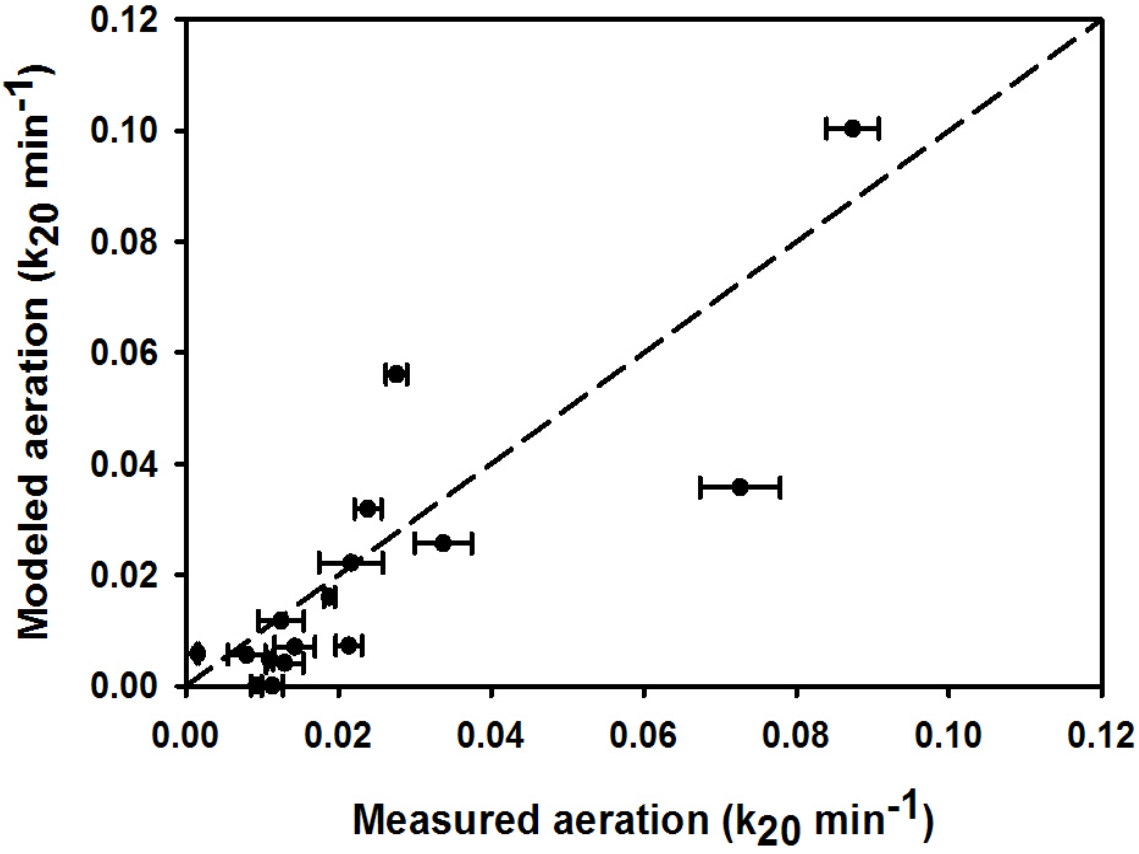

Figure 2.4 Correlation (Kendall Tau, p = 0.039) of measured aeration values and calculated aeration values using the equation from Tsivoglou and Neal (1976), both corrected at 20° C. Regression analysis resulted in an adjusted R^2 value of 0.72 and an equation of y = 0.1749x + 1.8375. Error bars represent standard error and the dashed line represents a 1:1 line.

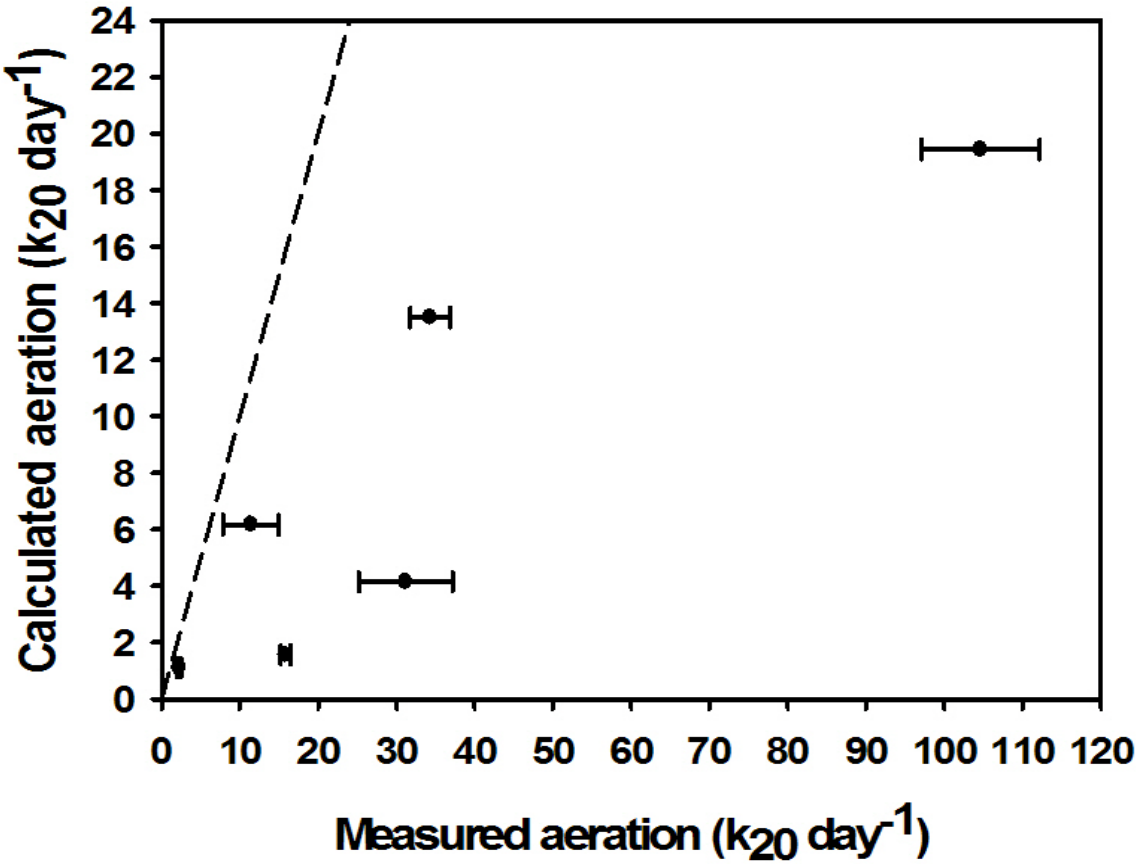

Figure 2.5 Ag North stream used to compare model parameters between 2 different scenarios of the model (full temperature corrected model and only aeration temperature corrected model). O_2 change in R (A) showed that not correcting respiration for temperature would result in a constant R rate. O_2 change in GPP (B) showed that temperature correcting only aeration had a greater swing in values than the full temperature corrected scenario. O_2 change in aeration (C) showed similar patterns as O_2 change in GPP, with temperature correcting only aeration being the most variable. The gray boxes represent nighttime and each point represents a 10 minute time period.

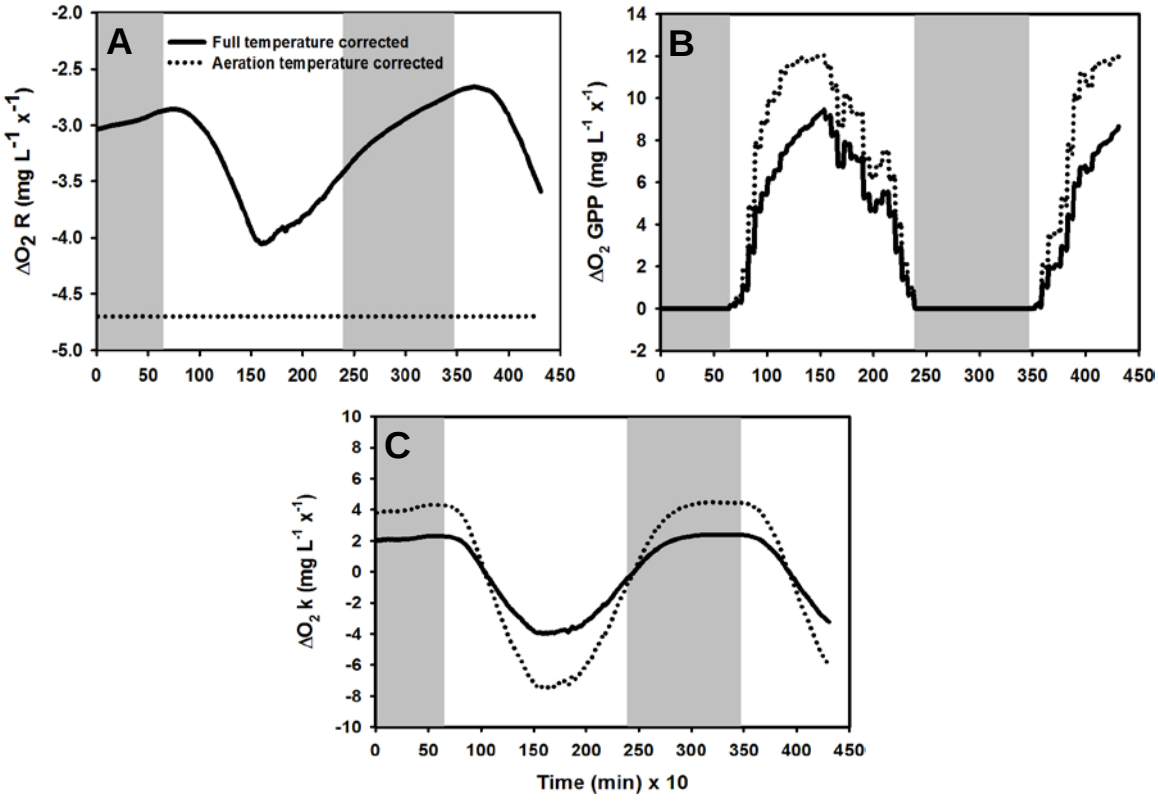

Figure 2.6 Change in O_2 between upstream and downstream stations of measured and modeled values from Ag North using the full temperature corrected model. Ag North is an example where O_2 was not increasing during the night. Gray boxes represent nighttime and each point represents a 10 minute time period. Modeled values closely resemble what was measured demonstrating how well the model fits measured values.

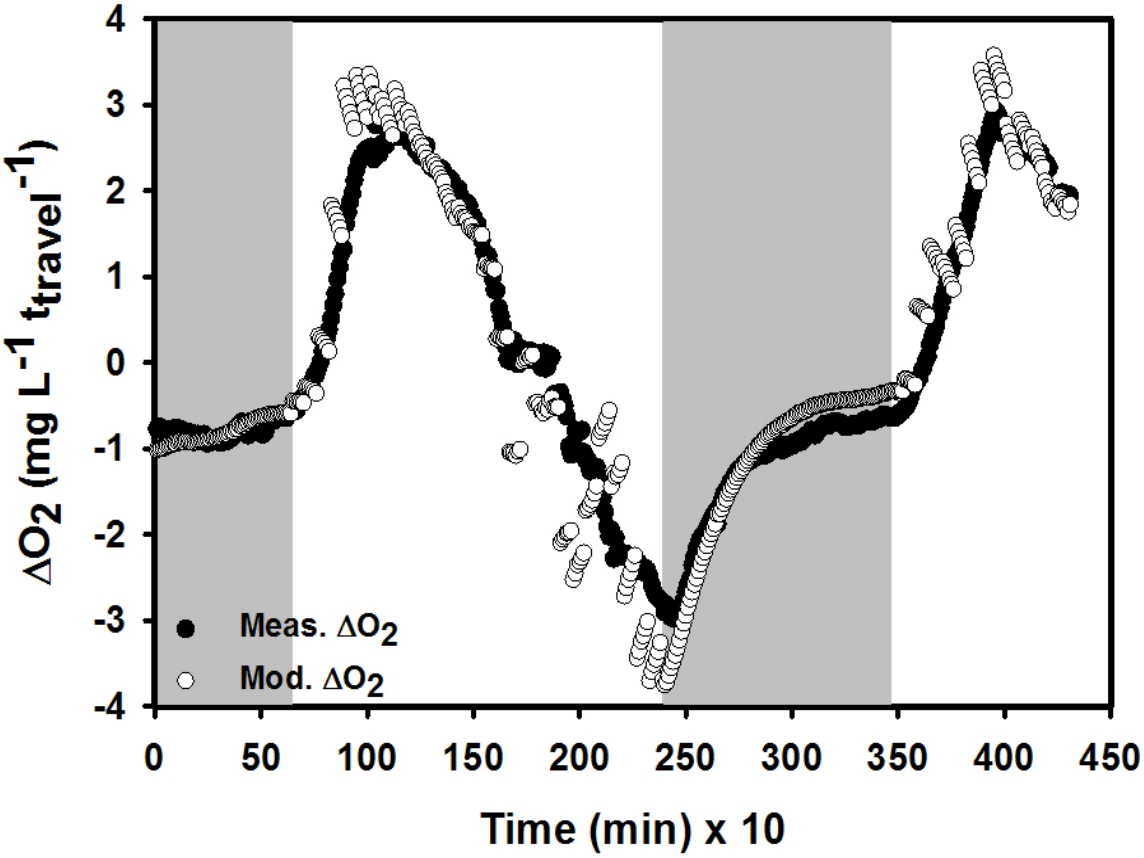

Figure 2.7 Change in O_2 between upstream and downstream stations of measured and modeled values from Natalie's Creek using the full temperature corrected model. Natalie's Creek represents a stream where O_2 was increasing during nighttime hours. Gray boxes represent nighttime and each point represents a 10 minute time period. Modeled values closely resemble what was measured demonstrating how well the model fits measured values.

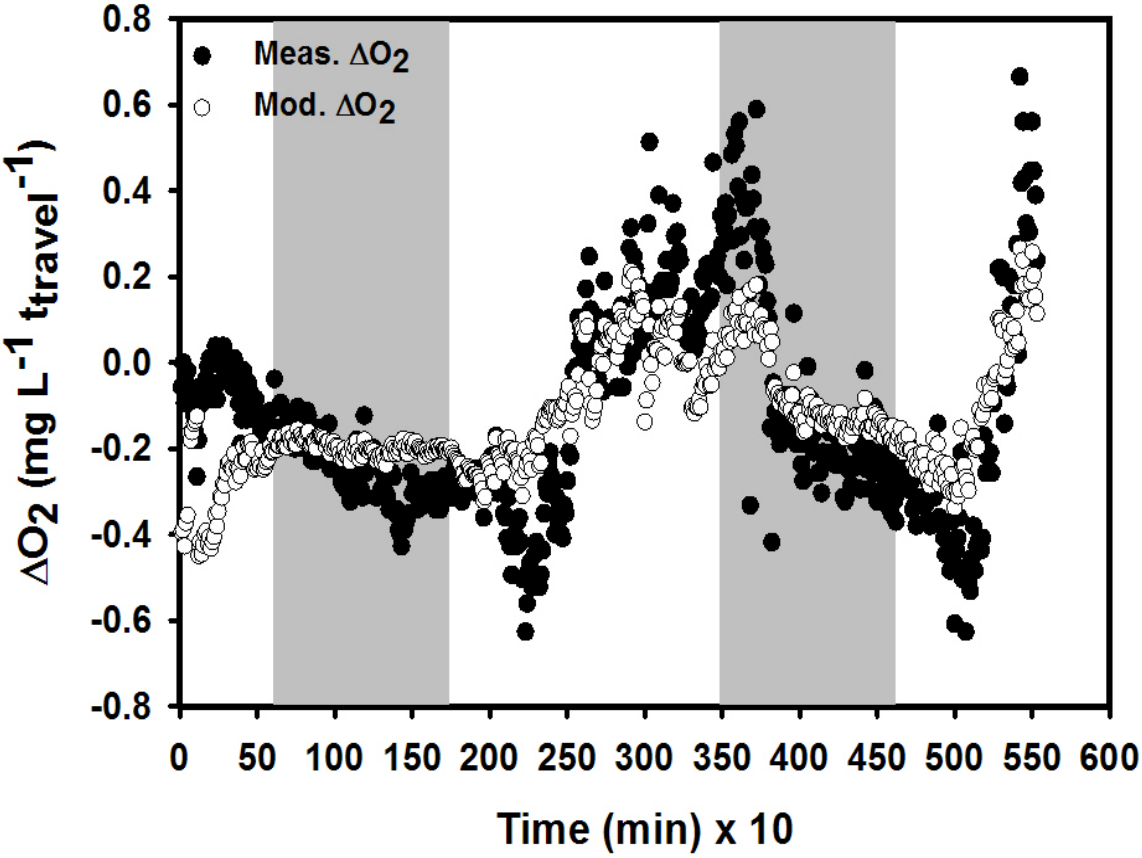

Figure 2.8 O_2 and temperature measurements from the downstream probe at Natalie's Creek show O_2 increasing during nighttime as temperature is decreasing throughout the night (A). Using the full temperature corrected model, aeration from Natalie's Creek decreases during the night as R also decreases during the night reaching the lowest rate just after sunrise (B). Gray boxes represent nighttime and each point represents a 10 minute time period.

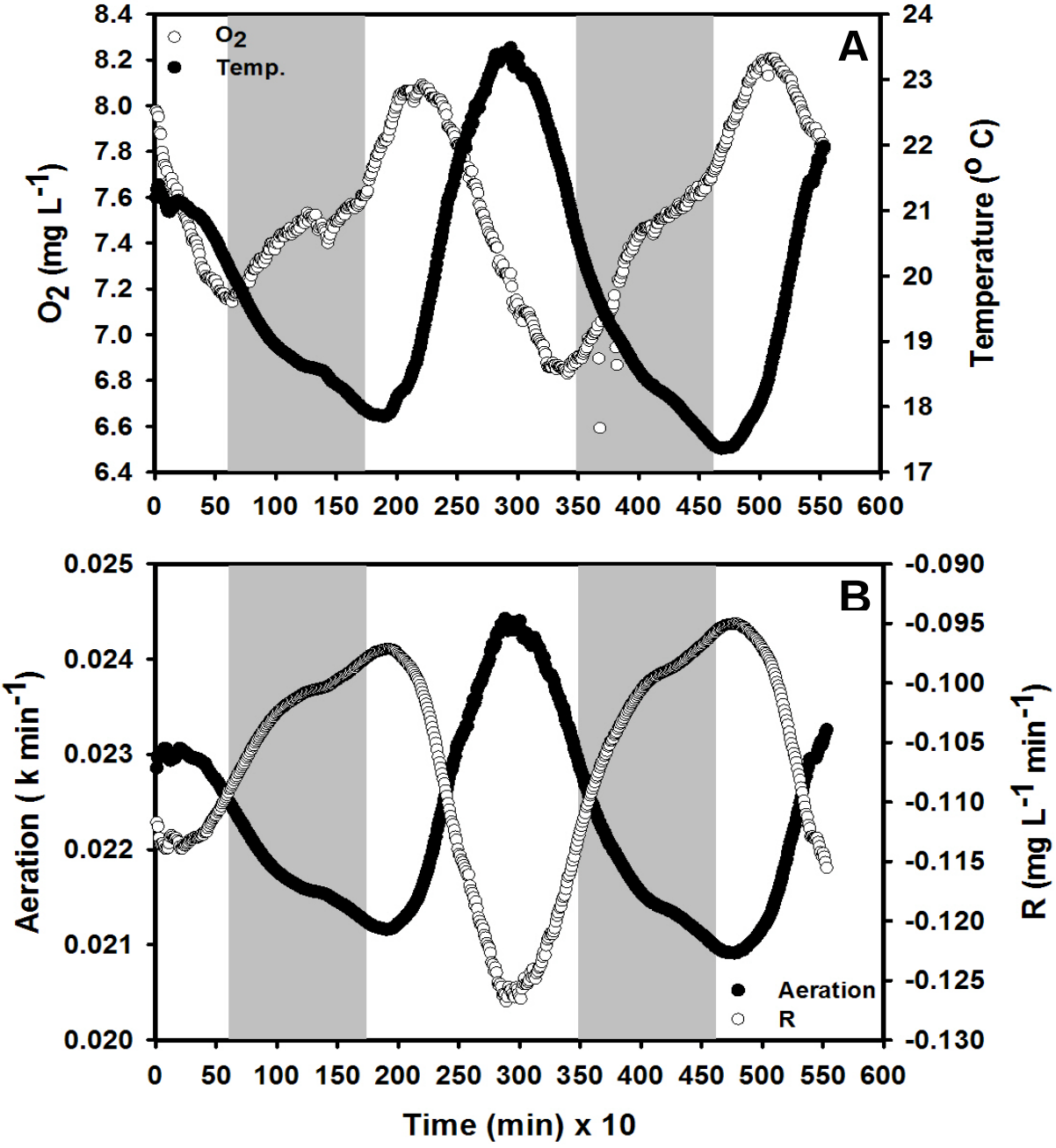

Table 2.1 Site characteristics for streams used in calculating aeration and metabolism.

Site	Min. T (^oC)	Max. T (^oC)	Avg. T (^oC)	Avg. w (m)	Avg. d (m)	Avg. v (m min^{-1})	Slope (%)	u (m^3 min^{-1})
Ag North	18.25	26.61	21.86	1.05	0.09	1.11	1.75	0.096
N04D	14.71	21.95	17.03	2.25	0.17	5.00	3.47	0.222
Campus	19.85	26.16	23.03	2.56	0.15	0.86	1.51	0.074
Natalie	17.38	24.47	20.07	0.92	0.07	1.55	2.74	0.030
Swine	18.82	23.51	20.22	1.33	0.13	2.80	1.87	0.377
Shane	13.64	18.26	15.49	2.19	0.13	1.20	3.78	0.113
K02A 06*	19.47	28.11	23.15	1.58	0.07	2.68	2.44	0.223
N04D 06*	14.98	24.45	19.06	1.13	0.07	1.45	1.38	0.097
K02A 07*	20.87	24.73	22.54	2.83	0.06	2.46	2.44	0.401
N04D 07*	20.16	27.94	23.16	1.23	0.08	1.24	1.38	0.121

*Sites used only for comparison of measured and modeled aeration

Table 2.2 Description of variables used for calculations and in the model.

Symbol	Units	Description	Measured/modeled/calculated
w	m	Average width	Measured
d	m	Average depth	Measured
v	$m\ min^{-1}$	Velocity	Measured
u	$m^3\ min^{-1}$	Discharge	Measured
x	m	Distance between stations (reach length)	Measured
k	min^{-1}	Aeration	Measured/Modeled
k_T	min^{-1}	Aeration at T ^oC	Calculated
t	min	Time between measurements	Measured
t_{travel}	min	Travel time between stations	Calculated
T_{avg}	^oC	Average temperature offset by travel time	Calculated
T_u	^oC	Temperature at upstream station	Measured
T_d	^oC	Temperature at downstream station	Measured
T_R	^oC	Temperature during aeration measurements	Measured
p	mm Hg	Barometric pressure not corrected for elevation	Measured
O_{2u}	$mg\ L^{-1}$	O_2 concentration at upstream station	Measured
O_{2d}	$mg\ L^{-1}$	O_2 concentration at downstream station	Measured
O_{2avg}	$mg\ L^{-1}$	Average O_2 concentration offset by travel time	Calculated
ΔO_2	$mg\ L^{-1}\ t_{travel}^{-1}$	O_2 change in water column during a time period	Calculated
ΔO_{2meas}	$mg\ L^{-1}\ x^{-1}$	O_2 change between stations offset by reach travel time	Calculated

O_2 $\%sat_{meas}$	%	O_2 % saturation from measured O_2 concentrations	Calculated
L	µmol q m^{-2} s^{-1}	Light	Measured
R	mg m^{-2} min^{-1}or mg L^{-1} min^{-1}	Respiration	Modeled
R_T	mg m^{-2} min^{-1}or mg L^{-1} min^{-1}	R at T ^oC	Modeled
R_{daily}	g m^{-2} d^{-1}	Daily R	Modeled
$Pmax_T$	mg m^{-2} min^{-1}or mg L^{-1} min^{-1}	Maximum photosynthesis at T ^oC	Modeled
α_T	(mg m^{-2} min^{-1}or mg L^{-1} min^{-1}) (µmol q^{-1} m^2 s)	Initial slope of the photosynthesis-irradiance curve at T ^oC	Modeled
GPP_{daily}	g m^{-2} day^{-1}	Daily gross primary production	Modeled
NEP_{daily}	g m^{-2} day^{-1}	Daily net ecosystem production	Modeled
SSE		Sum of squares of error	Calculated
ΔO_2 R_T	mg L^{-1} x^{-1}	O_2 change from R	Calculated
ΔO_2 GPP_T	mg L^{-1} x^{-1}	O_2 change from GPP at T ^oC	Calculated
ΔO_2 k_T	mg L^{-1} x^{-1}	O_2 change from k	Calculated
ΔO_{2mod}	mg L^{-1} x^{-1}	O_2 change from R, GPP and k	Calculated
ΔO_2 R_{T24avg}	mg L^{-1} x^{-1}	24 hour average of O_2 change from R	Calculated
ΔO_2 GPP_{T24avg}	mg L^{-1} x^{-1}	24 hour average of O_2 change from GPP	Calculated

Table 2.3 Equations used in the model along with a reference for the equation if taken from the literature.

Parameter	Equation	Reference
t_{travel}	x/v	
T_{avg}	$(T_d+T_u)/2$	
ΔO_{2meas}	$(O_{2d}-O_{2u}) x^{-1}$	
k_T	$k*((1.024\text{^}(T_{avg}-T_R)))$	Bott 2006
O_{2avg}	$(O_{2d}+O_{2u})/2$	
O_2 $\%sat_{meas}$	$((O_{2avg})//EXP(-139.3441+(157570.1/T_{avg}+273.15))-(66423080/((T_{avg}+273.15)\text{^}2))+(12438000000/$	
	$((T_{avg}+273.15)\text{^}3))-(862194900000/((T_{avg}+273.15)\text{^}4))))*(p/760)*0.998))*100)$	APHA 1995
ΔO_2 R_T	$(-1*((R_T/d/1000)*(1.045\text{^}(T_{avg}-20)*t_{travel})) x^{-1}$	Parkhill & Gulliver 1999
ΔO_2 GPP_T	$(Pmax_T*(1.036\text{^}(T_{avg}-20))*TANH(\alpha_T*(1.036\text{^}(T_{avg}-20))*L/(Pmax_T*(1.036\text{^}(T_{avg}-20))))*t_{travel}) x^{-1}$	Jassby & Platt 1976; Parkhill & Gulliver 1999
ΔO_2 k_T	$((-O_{2avg}+(O_{2avg}/(O2\ \%sat_{meas}/100)))*k_T*t_{travel})\ x^{-1}$	
ΔO_{2mod}	$(\Delta O_2\ R_T+\Delta O_2\ GPP_T+\Delta O_2\ k_T) x^{-1}$	
SSE	$(\Delta O_{2mod}-\Delta O_{2meas})\text{^}2$	
R_{daily}	$\Delta O_2\ R_{T24avg}*d/t_{travel}*60*24$	
GPP_{daily}	$\Delta O_2\ GPP_{T24avg}*d/t_{travel}*60*24$	
NEP_{daily}	GPP-R	

Table 2.4 Kendall Tau correlation analysis of measured aeration values for 6 Kansas streams compared to 19 empirical equations for aeration with significant results having a p-value < 0.05 and denoted by an asterisk (*).

Comparison	Kendall Tau	p-level
measured vs. modeled	0.600	0.091
measured vs. Padden and Gloyna 1971	0.600	0.091
measured vs. Dobbins 1965	0.600	0.091
measured vs. O'Connor and Dobbins 1958	0.200	0.573
measured vs. Krenkel and Orlob 1963	0.733	0.039*
measured vs. Cadwallader and McDonnell 1969	0.733	0.039*
measured vs. Parkhurst and Pomeroy 1972	0.867	0.015*
measured vs. Bennett and Rathbun 1972	0.333	0.348
measured vs. Churchill et al. 1962	0.733	0.039*
measured vs. Lau 1972	0.067	0.851
measured vs. Thackston and Krenkel 1969	0.600	0.091
measured vs. Langbein and Durum 1967	0.600	0.091
measured vs. Owens et al. 1964[a]	0.600	0.091
measured vs. Owens et al. 1964[b]	0.200	0.573
measured vs. Churchill et al. 1962	0.600	0.091
measured vs. Isaacs and Gaudy 1968	0.600	0.091
measured vs. Negulescu and Rojanski 1969	0.733	0.039*
measured vs. Bansal 1973	0.600	0.091

measured vs. Bennett and Rathbun 1972	0.200	0.573
measured vs. Tsivoglou and Neal 1976	0.733	0.039*

[a] Equation 18 in Owens et al. 1964

[b] Equation 19 in Owens et al. 1964

Table 2.5 Daily metabolism results (g O_2 m^{-2} d^{-1}) from the full temperature corrected scenario (measured aeration value and temperature corrected aeration, R, GPP) and the only aeration temperature corrected scenario for 6 Kansas streams. Ag North, N04D, and Swine were net autotrophic. Campus, Natalie, and Shane were net heterotrophic in both model scenarios.

	Temperature corrected			Not temperature corrected		
Site	R	GPP	NEP	R	GPP	NEP
Ag North	-6.94	7.03	0.09	-6.26	6.29	0.03
N04D	-6.08	10.17	4.09	-5.14	9.42	4.28
Campus	-2.43	1.37	-1.06	-2.31	1.35	-0.96
Natalie	-3.45	0.78	-2.67	-3.03	0.52	-2.51
Swine	-3.77	4.94	1.17	-3.45	4.56	1.11
Shane	-5.30	3.45	-1.85	-5.15	3.34	-1.81

Chapter 3 - Riparian woody expansion and subsequent restoration influences prairie stream metabolism

Abstract

Stream metabolism is central to stream ecosystem function. Tallgrass prairies and their streams are highly endangered ecosystems. Many remaining prairie streams are threatened by the encroachment of woody riparian vegetation. The main goal of this study was to determine the effects of canopy cover on prairie stream metabolism by comparing forested reaches to naturally open reaches and to reaches where woody vegetation was removed. The study was designed to test if removal of woody riparian vegetation restored stream metabolism to rates more characteristic of naturally open prairie streams. Metabolism was measured using the two-station diurnal method during 2006-2009 in Kings Creek, located on Konza Prairie Biological Station. Aeration rates were measured directly in the field using an inert gas and corrected for groundwater dilution by using a tracer dye or ion. Chlorophyll *a* concentrations and mass of filamentous algae were measured to assess potential differences in algal biomass between reaches with an open or closed canopy. Before the riparian vegetation removal, community respiration rates were greater with greater canopy cover (ANCOVA, $p = 0.001$). In the vegetation removal reaches, gross primary production was slightly greater after removal (ANCOVA, $p = 0.050$). Chlorophyll *a* concentrations were marginally significantly greater in open (naturally open and removal reaches) than closed canopy (ANOVA, $p = 0.057$) and differed significantly among seasons (ANOVA, $p = 0.031$). Woody expansion could increase community respiration, and increase prairie stream metabolism to be more net heterotrophic. An increase in canopy cover decreases benthic chlorophyll and potentially alters resources available to the stream food web.

Introduction

Role of canopy cover

The North American tallgrass prairie has been significantly altered as a consequence of human activity, resulting in the loss of more than 95% of prairie lands (Samson and Knopf 1994). In many areas where prairie has not been plowed for cropland, contemporary control of fires has encouraged the growth of woody vegetation. Historically, low-order prairie streams were commonly characterized by open canopies associated with riparian grass cover (Dodds et al. 2004). Now, the riparian vegetation of many of the few remaining tallgrass streams is becoming increasingly more similar to that found in deciduous forests. The rapid encroachment of woody vegetation into riparian areas on Konza Prairie Biological Station (hereafter Konza) has been well documented. Briggs et al. (2005) determined that woody vegetation cover near major stream channels increased approximately 70% from 1939 to 2002 on Konza. Much of this expansion has occurred along Kings Creek, which is a prairie stream with its watershed completely in tallgrass prairie and within the boundaries of Konza. Riparian cover of headwater streams influences downstream water quality parameters (Dodds and Oakes 2008). Furthermore, coarse allochthonous inputs from woody canopies are greater than in grassy riparian areas and dominated by carbon-rich leaves (Dodds et al. 2004; Stagliano and Whiles 2002). Thus, basic ecosystem structure could be changed fundamentally by woody expansion in the riparian zones of prairie streams.

Importance of prairie stream metabolism

Stream metabolism is related to downstream water quality because it is intimately tied to in-stream nutrient processing, and is an indicator of total activity and energy flux into the ecosystem. Gross primary production (GPP) and community respiration (CR) are processes of

stream metabolism. Net ecosystem production (NEP) is based on the balance of GPP and CR, and it is a measure of whole ecosystem production. Productivity can indicate the trophic status of a stream. Different portions of the food web depend upon heterotrophic microbial components and autotrophic components of the stream. Thus, it is important to understand both the CR as a measure of heterotrophic state, and GPP as a measure of autotrophic state (Dodds 2006). Understanding metabolic processes within the prairie stream ecosystem is imperative in protecting and attempts at restoration of this threatened system.

Objectives of study

We had three main questions: 1. How does woody vegetation encroachment, and thus, a change in canopy cover affect prairie stream metabolism? 2. What are the seasonal patterns of metabolism in prairie streams, and do these patterns vary with canopy type? 3. Does restoration of moderate-length reaches to open canopy restore prairie stream ecosystem function to be more similar to natural open canopy reaches?

We define restoration as having the goal of returning to a former ecosystem state. Since this is an ecosystem restoration, we are interested in returning the fundamental processes to rates similar to the native state (Bradshaw 1997). The removal of riparian woody vegetation was an attempt to bring the stream function back to the state of the native, naturally open canopy reaches. We tested if such restoration would be successful at the reach-scale level. We hypothesized that reaches with an open canopy would have greater GPP rates than reaches with a closed canopy, and that GPP would increase in the removal reaches after vegetation removal. It was also hypothesized that GPP would be greatest during summer when longer periods of sunlight would promote more primary production. Another hypothesis was that seasonal

changes in CR, related to loss of leaves from riparian deciduous trees, would be less pronounced in areas where riparian canopy was removed.

Methods

Study area

Our experiment was done during 2006-2009 in eight stream reaches in an intermittent prairie stream, Kings Creek, whose watershed is encompassed within Konza. Konza is a 3,487 ha tallgrass prairie that is in the northern part of the Flint Hills region near Manhattan, Kansas, in Riley County. It is owned by the Nature Conservancy and managed by the Division of Biology at Kansas State University. The Kings Creek watershed is located within the boundaries of Konza and is entirely in tallgrass prairie. Detailed site description has been published previously (Gray et al. 1998; Gray and Dodds 1998). Study reaches were located in two separate subwatersheds (Fig. 3.1), N04D and K02A/AL (hereafter referred to as K02A). N04D is continuously grazed by native American bison (*Bos bison*) and burned every 4 years. K02A is not grazed and is burned every 2 years. During the course of this study, N04D was burned in 2009 and K02A was burned in 2006 and 2008. AL (the site of 3 measurement reaches) was burned in 2009 and is not burned on a regular burn schedule.

Study areas consisted of four reaches in each subwatershed, stratified by differences in canopy cover that either occurred naturally or were altered experimentally (Fig. 3.2). The site designations indicate condition of each reach. The first letter indicates K02A (K) or N04D (N) as the subwatershed. The second letter indicates naturally open canopy (O), closed riparian canopy (C), or closed canopy removed experimentally (R). The third letter is needed because the closed canopy reaches in each subwatershed were located either upstream (U) or downstream (D) of the removal reach. The four reaches at N04D were consecutive. From upstream to

downstream the reaches were coded NCU, NR, NCD, and NO. The reach order at K02A from upstream to downstream was KO, KCU, KR, and KCD. KO and KCU were about 30 m apart, otherwise, reaches were contiguous. For the purpose of presentation of results, the removal reaches are referred to as NR-B and KR-B for before removal and NR-A and KR-A for after removal.

Riparian canopy cover differed among reaches. Both NO and KO were dominated by big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans*). These reaches also contained western ragweed (*Ambrosia psilotachya*), rough-leaved dogwood (*Cornus drummondii*), and numerous other rarer perennial forbs. NR-B, NCU, and NCD were dominated by American elm (*Ulnus americana*) and honey locust (*Gleditsia triacanthos*). KR-B, KCU, and KCD were dominated by bur oak (*Quercus macrocarpa*) and chinquapin oak (*Quercus muehlenbergii*). All closed canopy reaches in both watersheds had an understory that consisted of various grass and forb species. Following woody removal, NR-A was comprised mostly of Japanese brome (*Bromus japonicas*), western ragweed, and dogwood patches. KR-A consisted of more woodland understory species than NR-A. The most prominent species were Virginia creeper (*Parthenocissus quinquefolia*), buckrush (*Andrachne phyllanthoides*), and black snakeroot (*Sanicula canadensis*). Although riparian vegetation differed among the reaches, all eight reaches were similar in stream characteristics such as depth, width, velocity, and discharge (Table 3.1).

Experimental manipulation

Woody vegetation was removed from the stream to approximately 30 m away from the bank in the two removal reaches in December 2007 (Fig. 3.3, Fig. 3.4). The removal took place during the winter to ensure that the ground was frozen to minimize impact to the riparian area.

Larger trees were removed with a chainsaw, and smaller vegetation was removed with mechanical brush cutting and manual removal. After trees and large woody vegetation were cut down, the stumps were immediately sprayed with a dye (Liquid Dye Solution) and Roundup (super concentrate weed and grass killer) mixture to prevent future plant growth. Vegetation that was removed was pulled away from the stream and out of the removal area. The open canopy associated with removal of vegetation was maintained throughout the remainder of the study by mowing and manual clipping every winter. Prior to leaves falling from trees in the fall, a wire mesh fence with 1 cm holes was placed across the entire stream anchored into the bottom, on the upstream side of the removal reach. This fence was used to collect leaves and keep them out of the removal area, as we would not expect longer naturally open reaches to receive large subsidies of leaves from upstream. About once a week, leaves that had collected on the fence were gathered and placed in the water on the downstream side of the removal reach. After all of the leaves had fallen from the trees, the mesh fence was removed from the stream and leaves that had blown into the water were pulled out of the removal reaches to the bank of the stream.

Measurement of stream metabolism and aeration

Metabolism measurements occurred during the times when differences in canopy cover were expected to have the greatest influence. We measured metabolism at baseflow to indicate average conditions, and to allow aeration rates to apply in cases when they were not made at precisely the same time. Metabolism was measured at least once in each reach in the spring before full leaf coverage, at least once in the summer during full leaf coverage, and at least once in the fall/winter after the leaves fell for each year. Metabolism rates were measured 4-5 times a year for 4 years (2006-2009) using the 2-station upstream-downstream method (Marzolf et al. 1994; Young and Huryn 1998) to isolate metabolism of specific stream reaches. March, April,

and May measurements were classified as spring samples. June, July, August, and September were summer measurements, and October, November, and December were fall measurements.

Yellow Springs Instruments logging data sondes were used to record O_2 and temperature values every 10 minutes. Before deployment, all sondes were calibrated together in the field. Sondes were placed in the stream for 30 minutes to be certain that all sondes were the same temperature as each other and that each sonde was at the same temperature as the water, as calibration is sensitive to temperature of the O_2 sensor and the sonde body. Sondes were calibrated to air saturated water and allowed to log for 30 minutes. O_2 readings were checked, and calibration was repeated until all sondes gave the same results (within 3%) before deployment. At the end of deployment, sondes were again placed together at one station for 30 minutes. If the sondes did not read the same value post-deployment then the data were corrected assuming a linear drift in calibration over the period of measurement.

Aeration was measured in order to allow accurate modeling of whole-stream metabolism. Aeration measurements were conducted at baseflow during 2006, 2007, and 2009. Attempts to obtain aeration rates in 2008 failed due to gas chromatograph problems. Aeration was measured in the same reaches where diurnal O_2 measurements were done using a tracer gas (propane) and an inert tracer dye (rhodamine) or ion (bromide) in all 8 reaches (i.e., aeration rates were measured for every reach). Details of aeration determination methods can be found in chapter 2.

Replicates of gas samples were taken at each sonde placement point (i.e. top and bottom of each reach) so that an aeration rate could be determined for each of the 8 reaches where metabolism was measured. To collect gas samples, 40 mL of stream water were collected in a 60 mL syringe that had a 3-way stopcock attached. Water was slowly drawn into the syringe to avoid cavitation and subsequent degassing of the solution. Then 20 mL of helium gas (gas

chromatography carrier gas) were injected into the 60 mL syringe that contained the water sample. The syringe was shaken for 3 minutes to strip the tracer gas out of solution. The gas in the syringe was injected into an evacuated vial (vacutainer, 15 mL). The water from the syringe was analyzed for tracer ion concentration or dye fluorescence to account for the dilution on each sample.

Gas samples were analyzed as soon as possible (at most within 24 hours) with a gas chromatograph (Shimadzu GC-14A) equipped with a flame-ionization detector. There was no relationship between variation in discharge and aeration over time for the different locations (data not shown); therefore, we used the average aeration value for that reach (corrected for average temperature at time of measurement) since aeration was always measured at baseflow.

Estimation of metabolic rates

Every file of diel data from each sonde was first checked to make sure that the sonde had worked properly. Some files had to be discarded due to equipment malfunction. In some cases, files had to be discarded due to very rapid drops and subsequent rises in O_2, which were thought to be from an invertebrate respiring close to the O_2 sensor during deployment. Once raw diel data passed the first inspection, metabolic rate was modeled.

We estimated metabolism using a model that altered rates of GPP and CR to minimize the variance between measured and modeled O_2 values using the "Solver" function in Microsoft Excel 2003. The model (described in the previous chapter) corrected metabolic rates for temperature and incorporated measured light values. Sonde data used for each model run included temperature and O_2 which were measured in 10 minute intervals and were offset by the reach specific calculated travel time. Additional data required for the model included individual reach characteristics (length, depth, width, average velocity, and discharge), barometric pressure,

aeration rate, and light. Width measurements were made every few meters along the length of the reach and 5 depth measurements were taken across each width transect. Width and depth were measured during each aeration measurement. Light values were measured using a Li-Cor LI-1000 datalogger equipped with a PAR sensor. Light measurements were logged every 10 minutes (corresponding with the O_2 sonde measurements). The light sensor was placed on an elevated level object in an open-canopy area next to the stream in full sunlight to determine daily variation in light availability for primary producers.

The basic modeling approach was to calculate O_2 every 10 minutes as influenced by rates of GPP, CR, and aeration. Solver minimized the sum of squares of error (SSE) between modeled and measured values to find the best fit of our modeled O_2 to observed O_2 by changing the basic rates of GPP and CR (see chapter 1 for variables and equations used in model).

Every set of diel O_2 trends were modeled to provide an estimate of metabolic rates for each sampling event for each reach, which resulted in a total of 121 files. The fit of each model file was re-examined to make sure that every file appeared to be correct and resulted in realistic numbers. If files gave illogical results (e.g., low respiration rates with high GPP rates, or zero respiration rates) the file was removed. Problems with poor curve fitting were generally traced back to problems with O_2 measurements that were not obvious from observations of single station trends, but emerged as a result of comparing upstream and downstream differences in O_2. Modeling results from 10 of the 121 files were removed, leaving 111 files.

Other measurements: days since flood, % canopy, chlorophyll, and filamentous algal biomass

The number of days since flood was determined to assess potential effects on metabolism. An annual return interval (ARI) of 1.67 years is an event that moves cobble, and

was used to represent a flood in Kings Creek (Fritz and Dodds 2005). A U.S. Geological Survey gauging station (# 06879650) on Kings Creek is located directly downstream only a few km from both N04D and K02A. Discharge data from 1980-2009 indicated a discharge rate of 9.8 $m^3 s^{-1}$ had a 1.67 ARI and this was used as the minimum discharge to define a flood in Kings Creek. Discharge rates at this gauging station were examined for the study period of 2006-2009. Each flood was noted, and then the number of days that passed until the next metabolism measurement occurred was counted. The number of days since flood was not significantly correlated with metabolism (CR, GPP corrected to 20 ^oC, and NEP) in an analysis of covariance (StatSoft, Inc.) at the 0.05 significance level, so days since flood data are not presented.

Percent canopy was determined using a densiometer. Readings were based on the presence or absence of canopy cover visible in the densiometer and were taken every two meters in each reach during the summer months of 2007-2009. The percentage of presence to absence readings was used to determine the percent canopy. There were a total of nine readings for every reach that were averaged for reach-specific percent canopy (personal communication Jodi Vandermyde).

We noticed a potential increase in filamentous algae following the removal and this prompted the collected of chlorophyll and filamentous algal biomass after the vegetation removal occurred. Chlorophyll *a* concentration was measured during 2008 and 2009 (post vegetation removal). Five rocks were collected without bias from each reach three times per year. Collection times were in April, July, and November/December to reflect the greatest potential influences of canopy cover. All five rocks from each reach were placed in a known volume of 95 % ethanol in the same autoclave bag. The bag was then placed in a 78 ^oC water bath for 5 minutes and then placed in the dark for 12 hours (Sartory and Grobbelaar 1984). A projection of

rock area was determined by tracing the surface of each rock and comparing the scanned image to the image of a known area (SigmaScan 5, Systat Software Inc., San Jose CA, USA). Chlorophyll *a* concentration was measured either using a fluorometer (Turner model 112) or a spectrophotometer (Hitachi UV/VIS U-2900). The fluorometer had a filter set and lamp that did not allow for the interference of phaeophytin (Welschmeyer 1994). When the spectrophotometer was used, chlorophyll *a* was measured according to standard methods (APHA 1995) and corrected for phaeophytin and adjusted for absorption coefficients in ethanol as described by Sartory and Grobbelaar (1984).

Filamentous algae were collected during 2008 and 2009 at the same sampling times (although not in the exact same locations) as rocks were collected for chlorophyll measurements. Sample collection of filamentous algae consisted of tossing a 0.25 m^2 metal quadrat into the reach attempting to avoid bias, and manually gathering all of the filaments from within the quadrat. Five quadrats were taken from each reach per sampling event and the contents from each quadrat were kept separate. The filaments were dried in a drying oven at 60 °C for at least 24 h and an average biomass dry weight was obtained for each reach.

Statistical analysis

Statistical tests were conducted using the program Statistica (version 6.1, StatSoft Inc., Tulsa OK, USA). Nonparametric Kendall Tau correlation analysis was run using all 111 metabolism estimates as an exploratory method. Based on these results, GPP was corrected to 20 ^{o}C (Parkhill and Gulliver 1999) and will be referred to as GPP_{20}. Temperature correction was done to remove the seasonal temperature effects on GPP to independently analyze any canopy effects.

A series of analysis of covariance (ANCOVA) tests were conducted to simultaneously assess categorical (e.g., year and watershed) and continuous (e.g., days since flood, temperature, and percentage canopy cover) variables and control for effects that a Kendall Tau analysis could not control for. A two-way analysis of variance (ANOVA) of CR in the removal reaches was used to determine differences in rates before and after vegetation removal. An ANOVA was used to analyze the chlorophyll *a* results with chlorophyll as the dependent variable and season and percentage canopy cover as categorical variables. Filamentous algal biomass results were analyzed using a one-way ANOVA to determine if filament biomass significantly differed between open and closed canopy reaches. Reaches were assumed to be independent from each other. Thus, the statistical results may be subject to spatial autocorrelation, and should be viewed with caution.

Results

Respiration

CR and average temperature were not significantly correlated ($p = 0.525$), but GPP and average temperature were correlated ($p = 0.002$) across all 111 metabolism files (Table 3.2). Based on significant temperature effects, GPP_{20} was calculated as in Parkhill and Gulliver (1999) to remove the temperature effects on GPP and adequately analyze any seasonal canopy effects independent of temperature. CR rates did not have to be corrected to 20 ^{o}C to effectively analyze canopy effects.

Metabolism rates from both watersheds were combined and separated into before vegetation removal rates and after removal rates and averaged within season to estimate an average rate for spring, summer, and fall (Fig. 3.5). Rates were combined by watershed because ANCOVA on before and after removal results did not result in any significant correlations

between watershed with CR, GPP_{20}, and NEP ($p = > 0.05$). CR is presented in negative values (i.e. as O_2 consumption), so the more negative the CR rate, the greater the metabolic process rate. During the spring before vegetation removal, GPP_{20} was similar across reaches, regardless of canopy type, and the naturally open reaches were net autotrophic while the other closed canopy reaches were all net heterotrophic. After the removal, average spring rates revealed that the upstream closed reaches were net autotrophic. These closed canopy reaches could be net autotrophic due to measurements occurring in spring before full leaf coverage, so all reaches would receive similar amounts of sunlight. Generally, average summer metabolic rates increased after vegetation removal, but did not appear to be impacted by canopy cover. During the fall, after the removal, all reaches had greater CR rates, except for the removal reaches where CR decreased after vegetation removal. CR in the removal reaches was significantly different before and after vegetation removal when season was taken into account (Table 3.3), indicating that canopy affected metabolism. In spring and fall, respiration decreased in the removal reaches after removal. This occurred in spite of a trend toward increasing CR in closed canopy reaches after the removal as compared to before removal.

Gross primary production

We hypothesized that GPP_{20} rates would be the greatest during summer months when periods of sunlight were maximized. The maximum GPP_{20} rate during the course of the study was greatest in the summer for 7 of the 8 reaches (Fig. 3.6). However, GPP_{20} was extremely variable, and rates were high during other seasons as well. For example, NCD had a high GPP_{20} rate in the fall of 2006, and KCD had high values in the fall of 2008 and spring of 2009. NR, the only reach to have the maximum GPP_{20} rate during fall (2006), had the minimum rate 17 days prior.

It was also hypothesized that the removal reaches would have the greatest GPP_{20} rates after the removal. However, the maximum GPP_{20} rate for NR and KR occurred in different seasons and at different times in reference to the vegetation removal. The greatest rate for NR was before the removal during the fall in October of 2006. The minimum rate for NR was also in October 2006, and was about 5 times lower than the maximum. The maximum GPP_{20} rate for KR was 69 times greater than the minimum and was measured after the removal during the summer in July of 2009. The minimum rate for KR was measured before the removal during late summer in September of 2006. KR usually had greater GPP_{20} rates after the removal than before and also had the greatest difference between minimum and maximum rates across all 8 reaches.

Factors influencing metabolic rate

Before riparian vegetation removal, percentage canopy cover clearly impacted CR rates, as greater percentage canopy cover led to greater rates of CR (ANCOVA, $p = 0.001$) when comparing across all 8 reaches (Fig. 3.7; Table 3.4). However, during the period after vegetation removal, canopy did not significantly affect CR. The same pattern held true for NEP. Reaches with a greater percentage of canopy cover had a greater NEP rate (ANCOVA, $p = 0.001$) across all 8 reaches before the removal (Fig. 3.7; Table 3.5). After removal, canopy was not significant with NEP rates.

To assess the direct impact of removing canopy cover in the removal reaches, an ANCOVA of the 28 metabolism files from NR and KR was conducted to avoid the variance associated with the non-treatment reaches. The ANCOVA revealed that percent canopy was marginally significant with GPP_{20} ($p = 0.050$, Table 3.6), indicating that GPP_{20} was greater with less canopy cover. GPP_{20} for NR did not change significantly after the removal, however, GPP_{20}

for KR increased 5.6-fold after the removal (Fig. 3.8). The significance of the results across watersheds is driven by the difference in GPP_{20} rates before and after the removal at KR.

After the removal, we saw the amount of filamentous algae apparently increase in the removal reaches, and I observed more filaments at KR than NR. These observations prompted direct collection of filamentous algal biomass after the removal. After woody removal, open canopy reaches had more filaments than closed canopy reaches (Table 3.7). A one-way ANOVA showed a significant negative relationship between canopy cover (reaches designated as open or closed canopy) and filamentous algal biomass ($p = 0.006$).

Chlorophyll *a* was measured to determine if concentrations differed with canopy cover. Analyzing chlorophyll results from individual reaches with a factorial ANOVA showed that chlorophyll *a* did not differ significantly across all reaches ($p = 0.274$). Therefore, watersheds were combined and reaches were combined into just open or closed canopy (Fig. 3.9). A factorial ANOVA on the chlorophyll results of combined reaches and watersheds indicated a marginally significant difference, with greater chlorophyll in open than closed canopied reaches ($p = 0.057$). This test also showed that chlorophyll *a* did differ significantly among seasons ($p = 0.031$). During the spring, chlorophyll *a* concentration was 2.7 μg cm^{-2} greater in open canopy reaches than closed canopy reaches. Average chlorophyll concentrations were lower for summer, and open canopy reaches had an average chlorophyll *a* concentration of 2.3 μg cm^{-2} more than closed canopy. Open canopy reaches had the highest chlorophyll values in fall, and the average chlorophyll concentration was 1.5 times greater than closed canopy reaches.

Discussion

Temperature and metabolism

Temperature was similar across all 8 reaches during each individual round of metabolism measurements (e.g., adjacent reaches did not vary much from each other in any one round of sampling), signifying that temperature effects related to reach-scale canopy cover was not an important driver of whole-stream metabolism in this study. Furthermore, CR or NEP were not correlated with temperature overall, indicating other factors control these rates more strongly than temperature. However, it is possible that changes in canopy cover could cause a temperature response at the watershed-scale that was not evident at the reach-scale. For example, if canopy of a stream was completely open, water temperatures during the day could be higher than in forested streams with a closed canopy. Other studies have shown a clear effect of canopy removal on stream temperature (e.g., Moore et al. 2005).

The relationship between water temperature and whole-system metabolism was not straight forward in our study. GPP was related to temperature, but CR was not. Respiration rates of individual organisms increase with warmer temperatures, but complex communities may contain organisms with widely different responses to temperature, and overall rates of CR would not necessarily relate to changes in temperature. It also can be difficult to separate the effects of temperature from light on primary production rates (Wetzel 2001). Several studies have documented the significant effects of water temperature on community respiration rates in streams (Bott et al. 1985; Sinsabaugh 1997; Uehlinger et al. 2000). Conversely, a study of eight streams from different biomes in North America found that water temperature was not significantly correlated with GPP or CR (Mulholland et al. 2001). Temperature may be an important factor in some streams, but it is not a main driver of CR and NEP rates in Kings Creek.

How does woody canopy cover affect prairie stream metabolism?

Canopy cover can affect stream metabolism by altering the amount of allochthonous organic material in the stream or the amount of available sunlight. The 8 reaches in Kings Creek were dominated by heterotrophic processes since NEP was almost always negative, regardless of canopy type. These findings are in accordance with previous studies in Kings Creek that found this stream to be net heterotrophic, even in areas with open canopy (Dodds et al. 1996; Mulholland et al. 2001; O'Brien and Dodds 2010).

We do not know if CR in Kings Creek is controlled by the rates of allochthonous leaf input or dissolved organic carbon influx, but our data indicate some effect of leaf input related to canopy cover. If the latter were true, then the canopy removal over relatively short reaches should have negligible effects on CR. Before riparian vegetation removal, the greater the percentage canopy cover in Kings Creek, the greater the CR and the more NEP was pushed toward heterotrophy. Possibly, more time would be needed to see the same results after the removal. Closed canopy reaches had more leaf litter than open canopy reaches because fewer leaves fell in, and those that did were mostly removed. The organic matter from leaves increases microbial heterotrophic respiration (Roberts et al. 2007). An increase in heterotrophic respiration would increase CR, which includes both autotrophic and heterotrophic respiration. The significant relationship between percent canopy cover and CR before removal indicates that carbon input in Kings Creek is more from allochthonous material than autochthonous material.

When comparing all canopy types across the 8 reaches, canopy cover affected both CR and NEP and not primary production, suggesting that CR drives NEP more than GPP in Kings Creek. However, a study on metabolism in forested streams and clearfell streams (open canopy due to tree harvesting) found that CR was significantly greater in the clearfell streams than the

closed canopy forested streams (Clapcott and Barmuta 2010). That study used chamber estimates, which can underestimate the hyporheic contribution to whole-system metabolism and be heavily influenced by autotrophic respiration.

The hypothesis that GPP rates would be greater in open canopy reaches than closed canopy was supported for the removal reaches, but not across all 8 reaches. When comparing just the removal reaches before and after vegetation removal, the component of CR is small enough that the effects were not significant. However, the removal of vegetation in the removal reaches marginally significantly affected GPP_{20} rates.

There were minimal differences in primary production before and after the vegetation removal in reach NR. This is likely because of the orientation of NR and the south stream bank height (Fig. 3.3C), partially shading the stream in the afternoon. In watershed N04D, the study area of Kings Creek has a greater sinuosity than at K02A; leading to increased shading of the stream in particular reaches (Fig. 3.2). The significant relationship between canopy cover and GPP_{20} for the removal reaches was driven by the difference in primary production at KR. KR had a more elevated north bank than south bank, and this bank did not shade the stream as much as the high south bank at NR. Kings Creek flows in a more east to west direction in K02A than in N04D, where it flows more south to north (Fig. 3.2). Therefore, KR would get more direct sunlight than NR and influence the amount of primary productivity.

Greater primary production rates after the vegetation removal agree with the results of another study that compared a forested stream with a meadow stream. Primary production rates were greater in the open canopy meadow stream than in the closed canopy forested stream (Bott et al. 2006). In addition, studies of several small streams varying in surrounding land-use types and across biomes have also found light to be a driving factor in whole-stream primary

production (Mulholland et al. 2001; Bernot et al. 2010). An effect of increased sunlight associated with open canopy reaches was also evident with greater amounts of filamentous algae present in open canopy reaches than closed canopy reaches.

What are the seasonal patterns of metabolism in prairie streams?

Metabolism can vary seasonally (Wetzel 2001). Individual GPP_{20} rates from each reach were variable between seasons and among the same types of canopy cover, indicating seasonal effects in addition to temperature. Closed canopy reaches within the same watershed often gave very different rates for measurements on the same day indicating large spatial in addition to temporal variance (Fig. 3.6). Thus, metabolism is likely dependent on specific reach characteristics in addition to canopy cover. The maximum GPP_{20} rate for reaches in Kings Creek tended to occur in the summer; however, there were also high rates in the spring and fall.

Higher primary production rates in spring may be the result of algal communities starting to develop (i.e. communities are more productive) as temperatures increase from winter. This was somewhat evident in the current study as spring chlorophyll concentrations were greater than the summer concentrations. However, fall chlorophyll concentrations were also greater than the summer values. Periphyton communities (includes algae, cyanobacteria, and heterotrophic bacteria) commonly peak in early spring or fall (Cushing 1967; Flemer 1970, Gumtow 1955; Marker 1976). Mulholland et al. (2001) measured metabolism in Kings Creek in April of 1998 and observed that the periphyton communities were already starting to senesce. This indicates that the algal communities could have been more productive prior to the onset of senescence (very early spring).

Chlorophyll *a* concentrations, which serve as a surrogate for biomass of primary producers, varied seasonally and were slightly affected by canopy cover. Chlorophyll *a* was

marginally significantly greater in open canopied reaches than in closed canopied reaches and significantly differed among seasons. Seasonal differences could be dependent on the quantity of grazers present during different times of the year. Chlorophyll *a* concentrations were lowest in the summer, which could be a result of floods, as most floods in Kings Creek occur in late spring or early summer. Robinson and Minshall (1986) have documented seasonal difference in chlorophyll *a*. They found greater chlorophyll *a* concentrations in fall than in summer and greater concentrations in an open canopy reach than a closed canopy reach. Seasonal changes in algal communities, and thus chlorophyll *a*, contribute to variability in seasonal GPP_{20} rates in Kings Creek.

In addition to variable primary production rates, CR was also highly variable across seasons and years. It was hypothesized that seasonal changes in CR would be less pronounced in the removal reaches after the removal because of the decrease in leaf input to the reach. This was not evident when temporal trends in CR rates were observed. Similar to the patterns of GPP rates, CR from the two closed canopy reaches in both watersheds were often very different for measurements from the same day, demonstrating substantial spatial variance in CR rates.

Metabolic rates from the eight reaches in Kings Creek, albeit variable, do fall within the range of metabolic rates from streams where metabolism is commonly measured. Metabolism was measured in 72 streams across eight regions in North America that varied in surrounding land-use (Bernot et al. 2010). Of the 72 streams, 24 were reference streams that ranged from forested to grassland and included Kings Creek. Bernot et al. (2010) found the reference streams to be more net heterotrophic than streams surrounded by urban areas or agricultural land. The 24 reference streams had a GPP range of 0.05 to 3.90 g O_2 m^{-2} d^{-1} and a CR range of -0.40 to -23.10 g O_2 m^{-2} d^{-1}. The average rates for GPP and CR from these reference streams were 1.20 and -

6.93 g O_2 m^{-2} d^{-1}, respectively. The 8 reaches in Kings Creek from the current study had a GPP range of 0.01 to 10.53, with an average of 1.91 g O_2 m^{-2} d^{-1}. The CR range for the 8 reaches in Kings Creek was -0.01 to -17.69, with an average of -4.73 g O_2 m^{-2} d^{-1}. Although the GPP range for the current study was greater than the range from the 24 reference streams, the range of the current study is within the range of GPP from 72 streams across regions (range of 0.05 to 16.20 g O_2 m^{-2} d^{-1}, Bernot et al. 2010). The average GPP rate from the current study is comparable to the average rate from the 24 reference streams. The CR range for the current study was within the CR range of the reference streams measured by Bernot et al. (2010).

Not only does metabolism vary seasonally, as would be expected due to changes in canopy cover in closed canopy streams, but metabolism can be highly variable on a day-to-day basis (Roberts et al. 2007). One reason for day-to-day variation is because of the constant change in cloud cover, which affects the amount of available sunlight. For this study, metabolism was only measured on days when cloud cover was predicted to be less than 30% according the National Weather Service, and we used no data from cloudy days. We did not measure metabolism frequently enough to characterize day-to-day variation.

Does restoration of reaches to open canopy represent naturally open reaches?

It is important to assess whether or not the changes that woody vegetation encroachment impose on prairie stream metabolism can be reversed with the removal of riparian canopy cover. During this study, it appeared that the removal reaches moved toward CR rates measured in the naturally open canopy reaches. In the removal reaches after the removal, CR rates increased after the removal, bringing rates closer to those measured in the naturally open reaches. Average GPP rates did not indicate that removal reaches mimicked the naturally open reaches. This study was only conducted for two years post vegetation removal, and it is possible that it may take

much longer to see the full effects of removing riparian canopy cover and restoring prairie stream reaches. Longer removal reaches may also have been required to see significant effects of canopy restoration. One effect of canopy removal that was immediately evident was the visual appearance of greater amounts of filamentous algae in the removal reaches, which was more similar to the reaches with a naturally open canopy.

Conclusion

The effects of canopy cover on GPP were only significant for the removal reaches and not when comparing all types of canopy cover (including closed canopy and naturally open canopy). It is possible that the reaches were too short or metabolism was not measured frequently enough. These results indicate that light might not limit GPP as much as other factors in this system (e.g., nutrients). Nutrient bioassays have demonstrated both reaches to be strongly limited by N and P (Johnson et al. 2009). Despite the reasons for weaker GPP results than expected, this study indicates that the encroachment of woody vegetation on prairie streams could alter CR and NEP, both key features of ecosystems. The results also indicate that woody riparian vegetation removal leads to CR rates that are more like rates in naturally open than in closed canopy reaches.

The endangerment of prairie streams makes continued research on these systems even more vital. Of the human activities impacting prairie streams, management practices that lead to encroachment by woody vegetation are yet another threat to an already rare ecosystem type. Prairie streams are typically headwater streams, therefore, it is important to preserve them to minimize the impact that changes in headwaters could have on downstream water quality. In the absence of additional data, the precautionary principle would dictate that maintaining ecosystem function of prairie streams requires in part maintenance of an open canopy, further research is

necessary to elucidate the full efficacy of woody riparian removal as a restoration technique for prairie stream ecosystem structure and function.

Figure 3.1 Map of Kansas (top) showing the location of Konza Prairie Biological Station within Riley county (marked with a star). The two study watersheds, N04D and K02A are highlighted to show their orientation to each other (bottom). Maps are courtesy of Adam Skibbe.

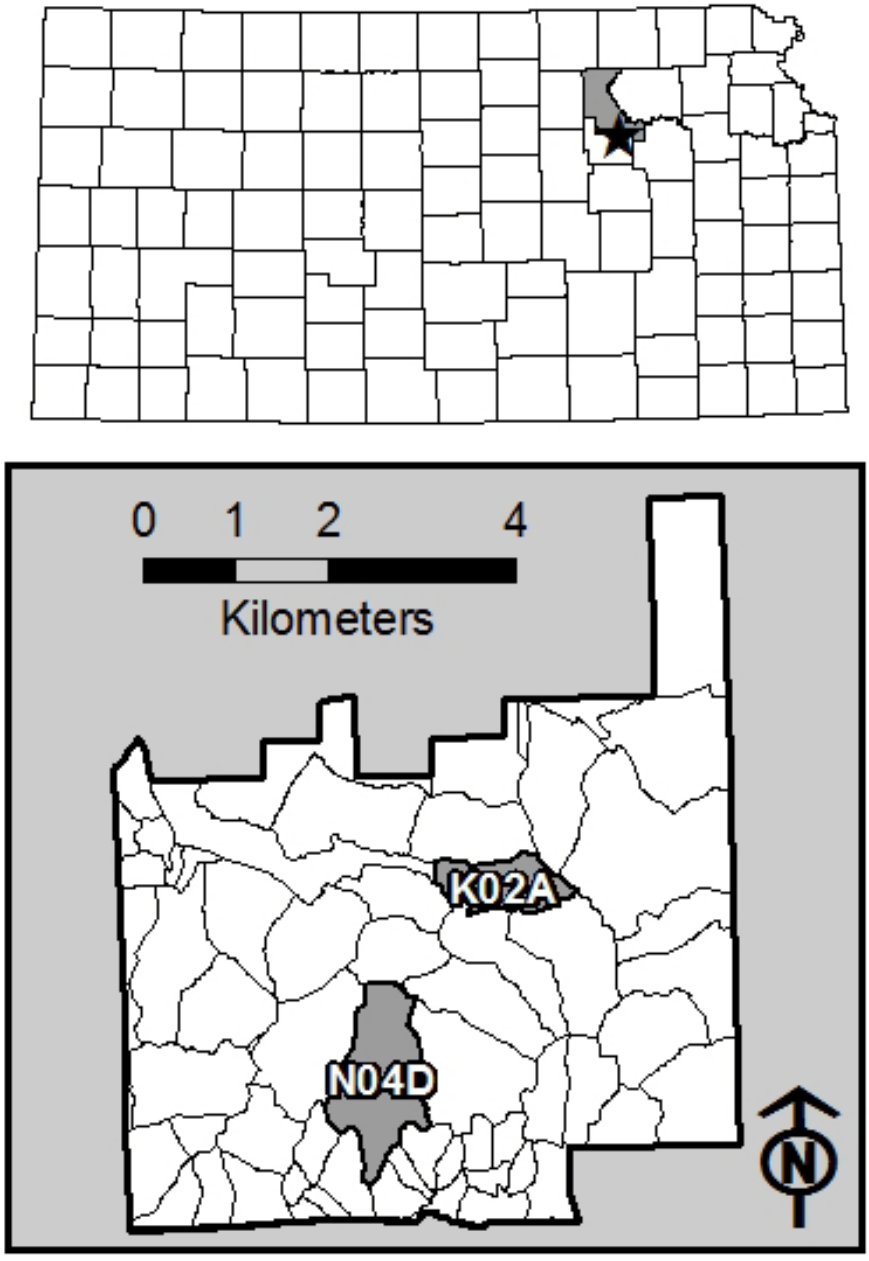

Figure 3.2 Aerial photographs of study sites on Konza Prairie Biological Station. Image on the left is of watershed N04D and image on the right is of study reaches located in watersheds K02A/AL. The stream channel is marked with a dashed line and individual reaches are represented by circles placed in the midpoint of the reach along with the reach code. Images are courtesy of Adam Skibbe.

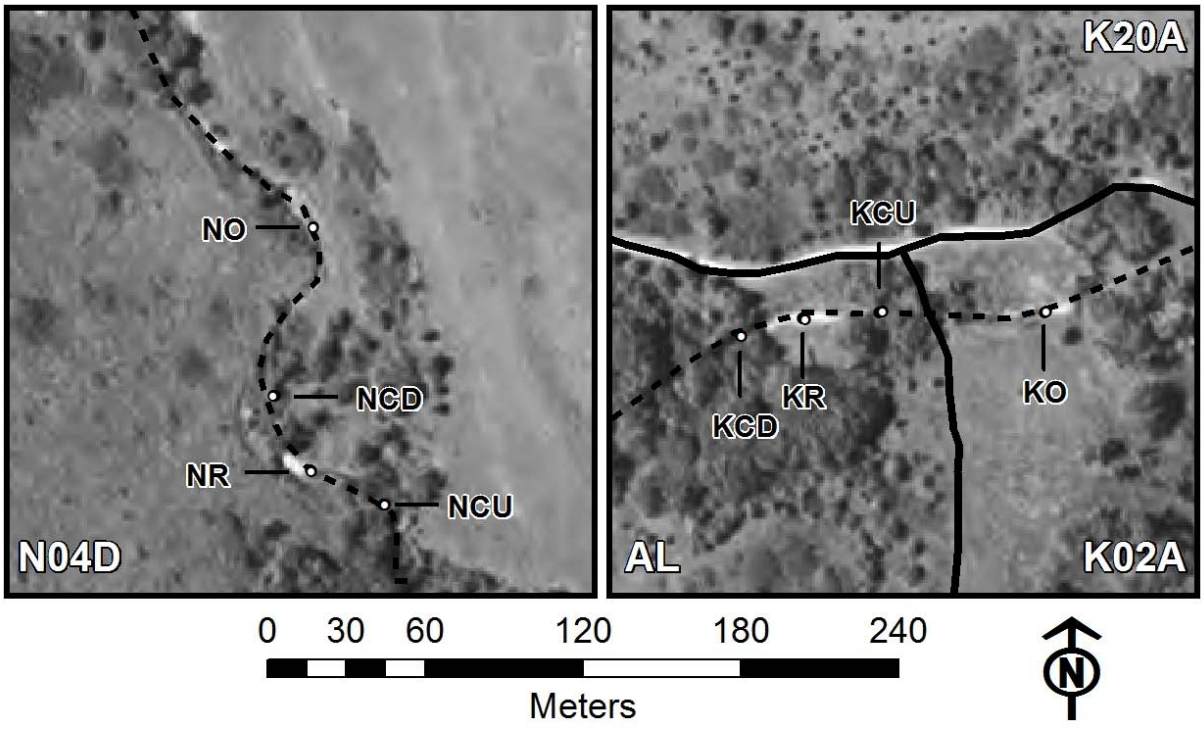

Figure 3.3 Removal reach at N04D (NR) from before vegetation removal in August 2007 (A), and after removal in August 2008 (B). Picture of removal reach at N04D immediately following woody vegetation removal in December 2007 (C).

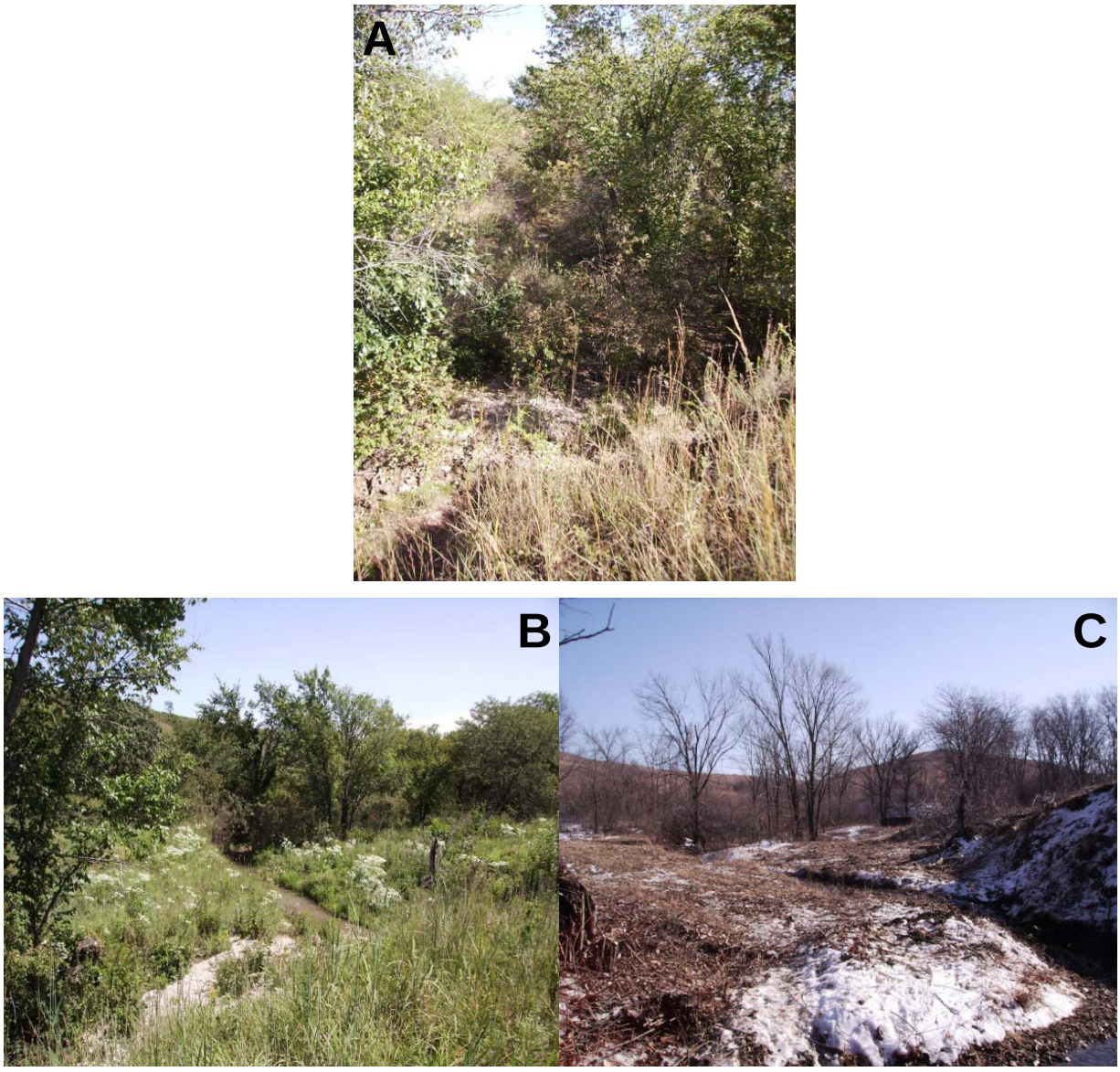

Figure 3.4 Removal reach at K02A (KR) from before vegetation removal in August 2007 (A), and after removal in August 2008 (B). Stream in (A) and (B) runs below the front line of vegetation in (A). Picture from August 2008 standing in removal reach looking downstream (C).

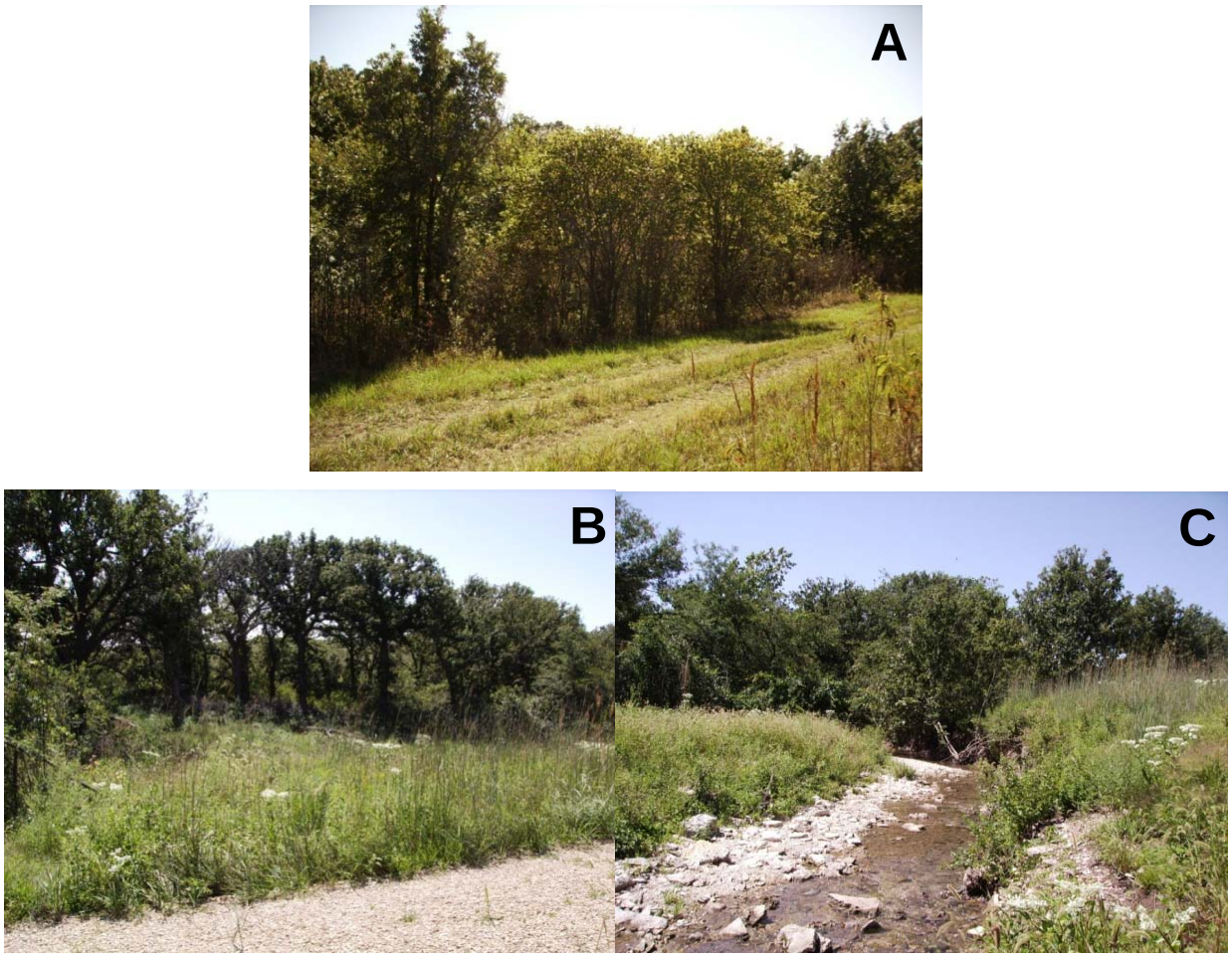

Figure 3.5 CR, GPP_{20}, and NEP rates measured from watersheds N04D and K02A in Kings Creek. Metabolism rates were separated into before removal rates (2006-2007) and after removal rates (2008-2009) and averaged for each season. Rates were combined by watershed (ANCOVA, $p > 0.05$ for watershed) to get an average rate for spring before removal (A), spring after removal (B), summer before removal (C), summer after removal (D), fall before removal (E), and fall after removal (F). Error bars represent standard error.

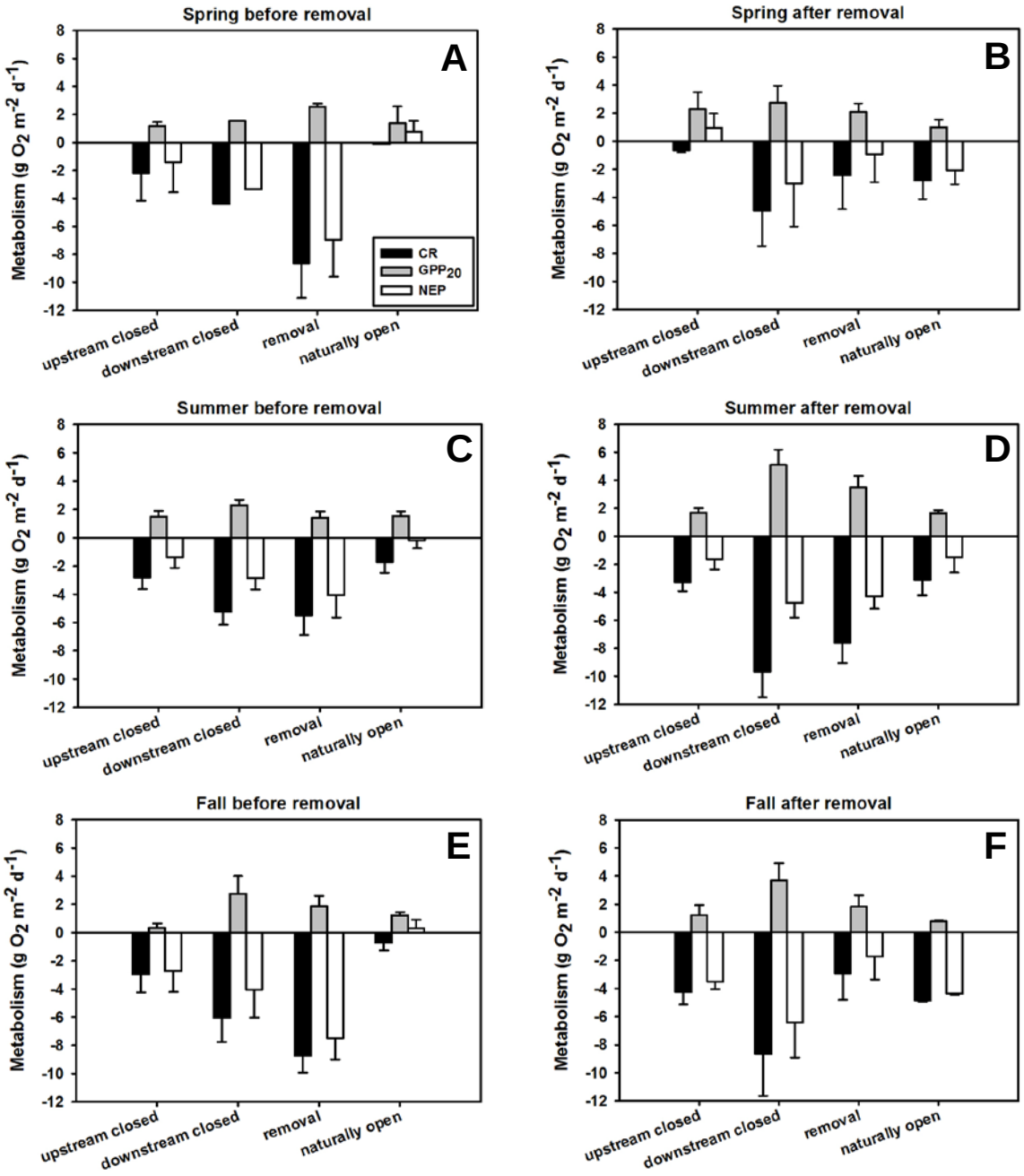

Figure 3.6 GPP_{20} rates measured during 2006-2009 for all 8 study reaches from watersheds N04D and K02A in Kings Creek: NO and NR (A), NCU and NCD (B), KO and KR (C), and KCU and KCD (D). The black arrow indicates when the vegetation removal occurred (December 2007). Reaches missing a rate for a sampling date was due to equipment failure.

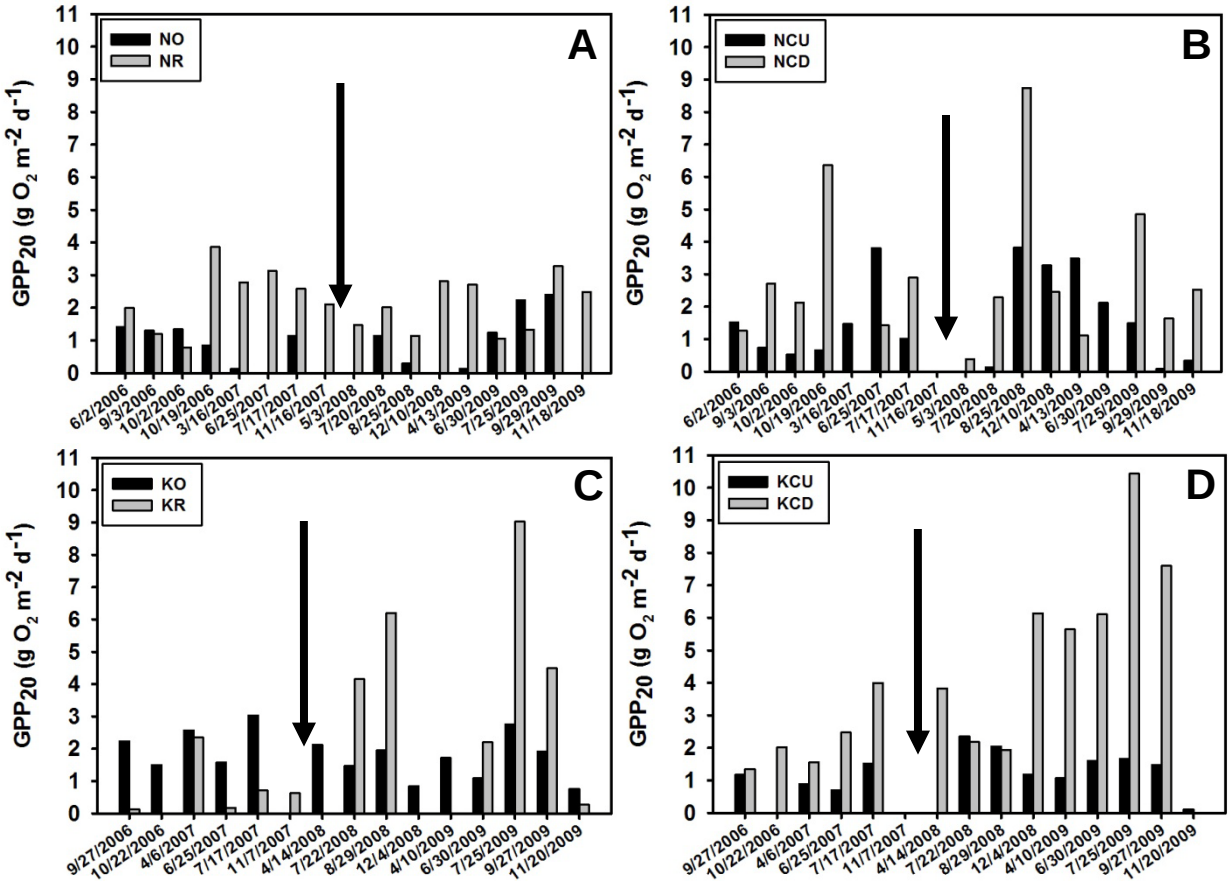

Figure 3.7 Average CR before the removal (A), CR after the removal (B), NEP before removal (C), and NEP after removal (D) with percent canopy cover for all 8 reaches in Kings Creek. Before riparian vegetation removal, the greater the canopy cover the greater the CR rate (ANCOVA, p = 0.001) and NEP increased with percent canopy cover (ANCOVA, p = 0.001). The removal reach is denoted as measurements before the vegetation removal (NR-B and KR-B) and measurements after the removal (NR-A and KR-A). Error bars represent standard error.

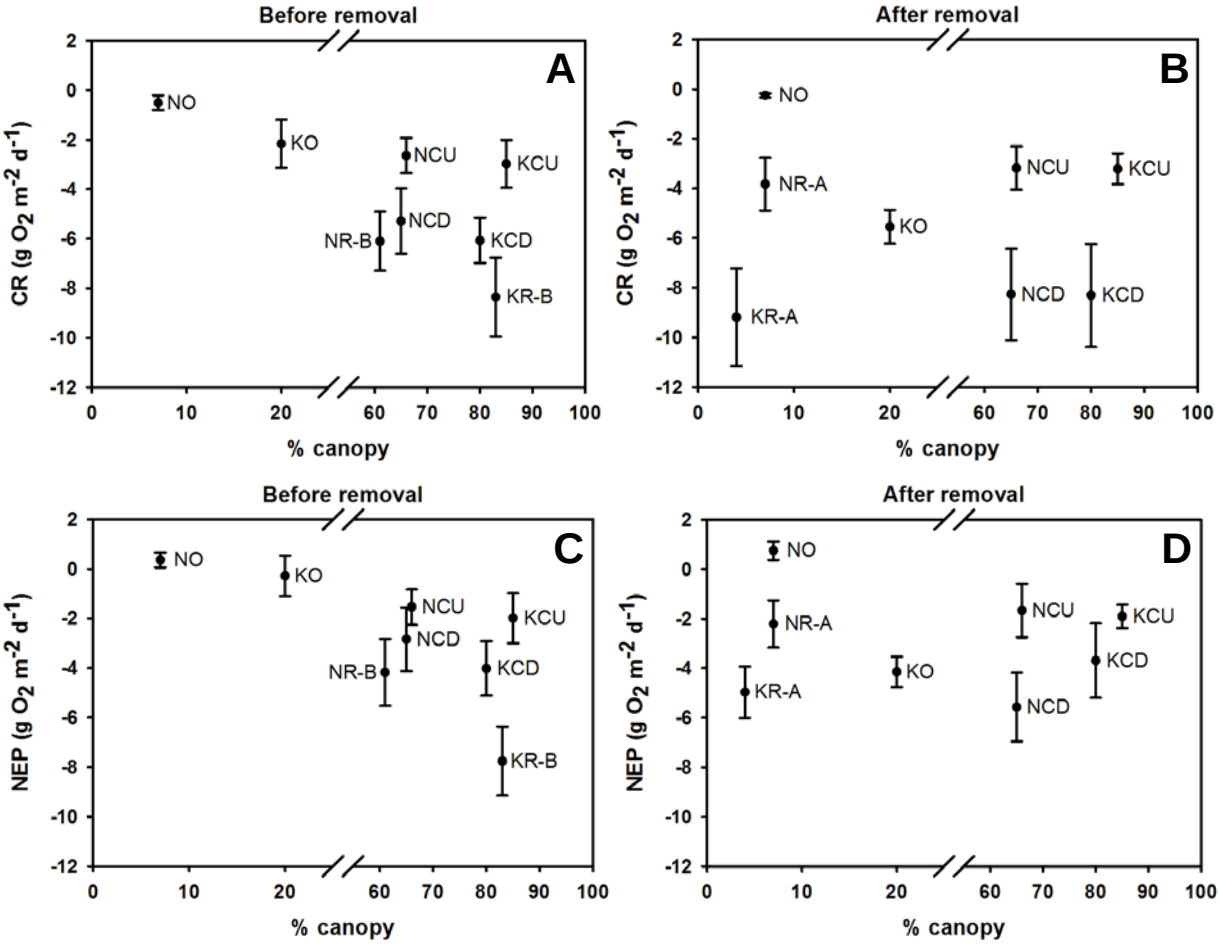

Figure 3.8 Average GPP_{20} rate for just the removal reaches (NR and KR) in Kings Creek. The percent canopy cover before/after the removal is displayed next to the reach code. Error bars represent standard error. Percent canopy was significant with GPP_{20} (ANCOVA, p = 0.050). This was driven by the difference in the GPP_{20} rate before and after the removal at KR.

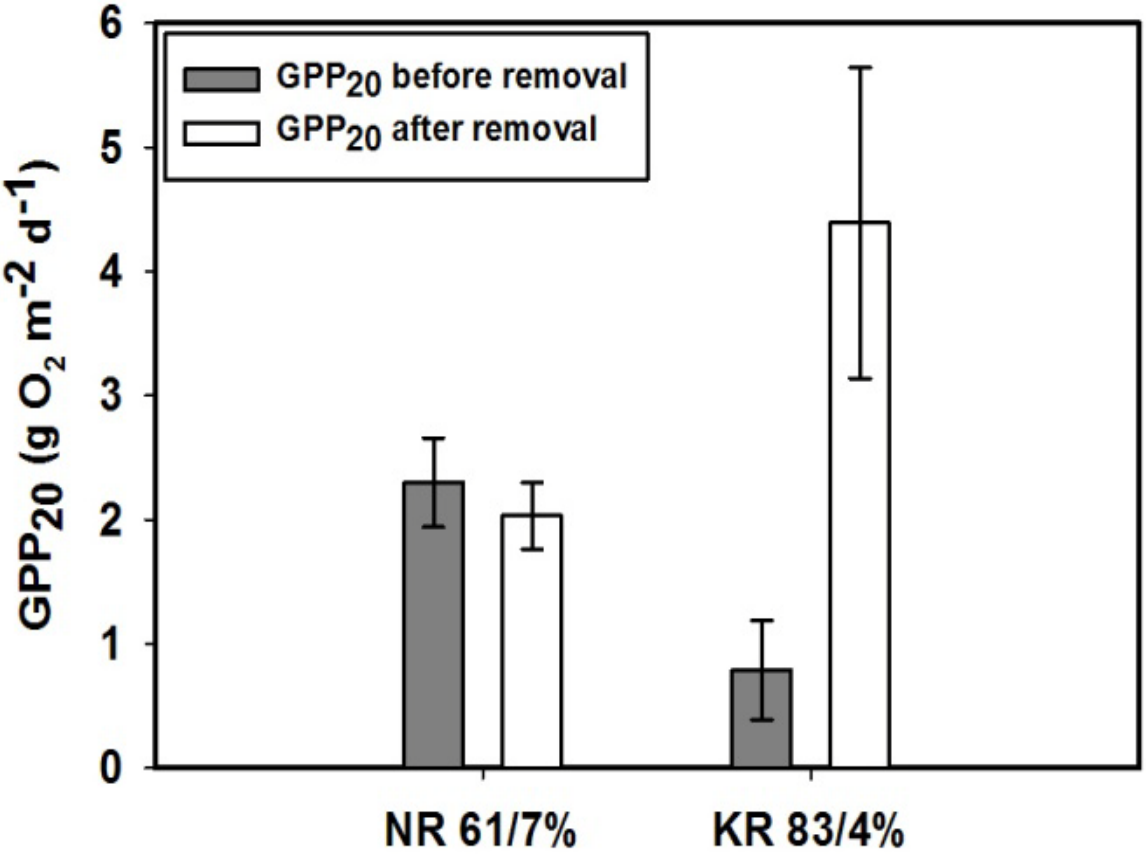

Figure 3.9 Average chlorophyll *a* concentration for all reaches combined into open canopy (NO, NR, KO, and KR) and closed canopy (NCU, NCD, KCU, and KCD). Error bars represent standard error. Rocks were collected for chlorophyll *a* analysis in April, July, and November/December of 2008 and 2009 (after removal). Open and closed canopied reaches differed marginally (factorial ANOVA, p = 0.057). Chlorophyll *a* results differed significantly among seasons (factorial ANOVA, p = 0.031).

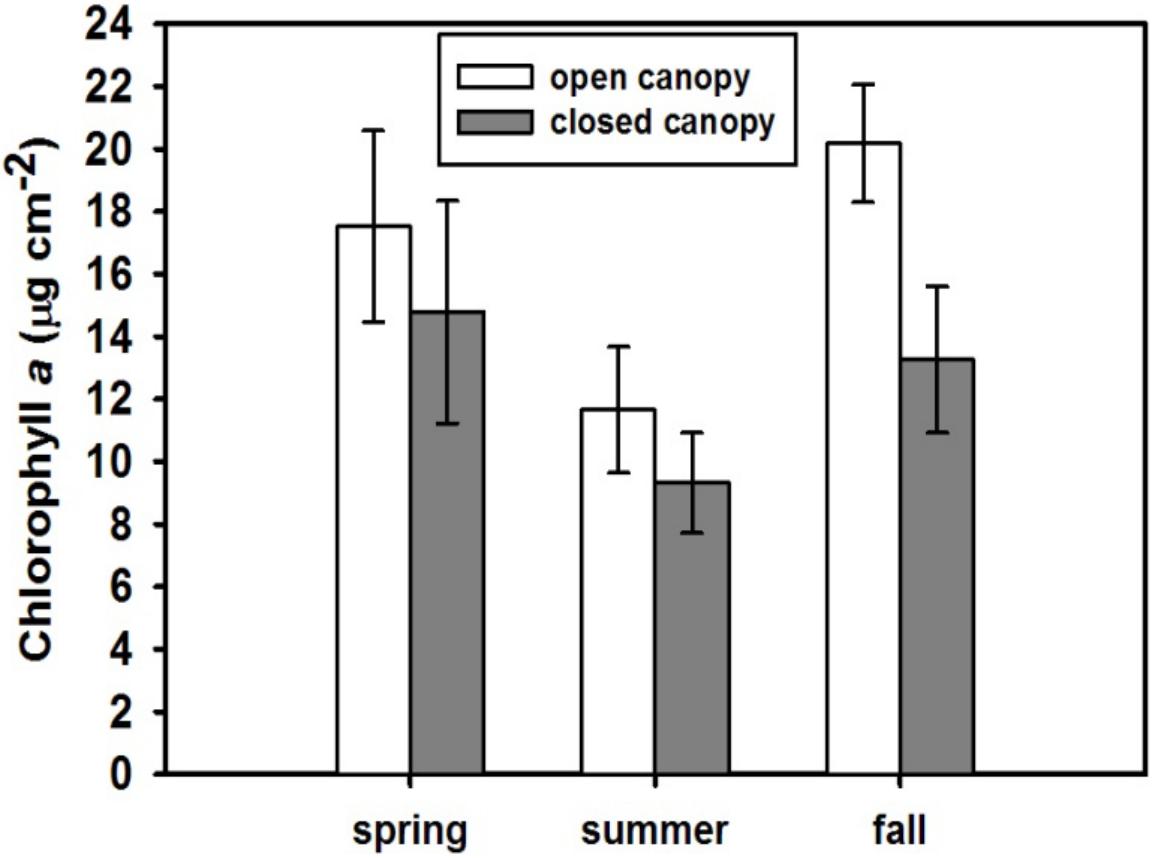

Table 3.1 Average values of site characteristics for stream reaches during the study period (2006-2009). Aeration values (k) were corrected to 20 ^{o}C. Before and after vegetation removal percent canopy values are displayed for the removal reaches (NR and KR).

Reach	Length (m)	Depth (m)	Width (m)	Aeration k_{20} (min^{-1})	Velocity (m min^{-1})	Discharge (m^3 min^{-1})	Canopy (%)
NCU	22.5	0.08	1.33	0.024	2.76	0.26	66
NR	33	0.11	1.45	0.044	1.83	0.28	61/7
NCD	36	0.10	1.11	0.050	4.07	0.47	65
NO	63.5	0.08	1.72	0*	2.53	0.31	7
KO	29	0.13	2.45	0.019	1.18	0.36	20
KCU	28.5	0.04	3.35	0.031	1.57	0.35	85
KR	35.5	0.12	3.06	0.027	2.71	0.89	83/4
KCD	27	0.20	4.02	0.030	1.54	1.12	80

*k was measured during 2 different years with no significant difference in gas values between top and bottom of reach

Table 3.2 Kendall Tau correlation analysis of average temperature (^{o}C) compared to 111 metabolism measurements from 8 reaches in Kings Creek with significant results having a p-value < 0.05 and denoted by an asterisk (*).

Comparison	Kendall Tau	p-level
temperature vs. CR	-0.041	0.525
temperature vs. GPP	0.196	0.002*
temperature vs. NEP	0.034	0.596

Table 3.3 Two-way ANOVA results from 2 removal reaches (NR and KR) with CR as the dependent variable and season and BR/AR (before removal/after removal) as categorical variables. Significant results had a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	701.465	1	701.465	46.214	< 0.001*
season	4.762	2	2.381	0.157	0.856
BR/AR	53.393	1	53.393	3.518	0.074
season*BR/AR	105.995	2	52.997	3.492	0.048*
error	333.930	22	15.179		

Table 3.4 ANCOVA results from 8 reaches in Kings Creek for 2006 and 2007 metabolism (before riparian vegetation removal) with CR as the dependent variable, season and watershed as categorical variables and days since flood, temperature, and % canopy as continuous variables. Significant results had a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	1.968	1	1.968	0.223	0.639
days since flood	8.419	1	8.419	0.954	0.335
temperature	1.103	1	1.103	0.125	0.726
% canopy	125.302	1	125.302	14.196	0.001*
season	41.285	2	20.642	2.339	0.110
watershed	1.572	1	1.572	0.178	0.675
season*watershed	8.314	2	4.157	0.471	0.628
error	344.225	39	8.826		

Table 3.5 ANCOVA results from 8 reaches in Kings Creek for 2006 and 2007 (before riparian vegetation removal) with NEP as the dependent variable, season and watershed as categorical variables and days since flood, temperature, and % canopy as continuous variables. Significant results had a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	0.009	1	0.009	0.001	0.974
days since flood	4.120	1	4.120	0.478	0.493
temperature	0.145	1	0.145	0.017	0.897
% canopy	115.026	1	115.026	13.349	0.001*
season	42.461	2	21.231	2.464	0.098
watershed	3.929	1	3.929	0.456	0.504
season*watershed	9.533	2	4.766	0.553	0.580
error	336.064	39	8.617		

Table 3.6 ANCOVA results from 2 removal reaches (NR and KR) with GPP_{20} as the dependent variable, watershed as a categorical variable, and days since flood and % canopy as continuous variables. Significant results had a p-value ≤ 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	122.300	1	122.300	36.424	< 0.001*
days since flood	0.051	1	0.051	0.015	0.903
% canopy	14.271	1	14.271	4.250	0.050*
watershed	3.672	1	3.672	1.094	0.306
error	80.584	24	3.358		

Table 3.7 Average dry mass (DM) weight of filamentous algae collected during April, July, and November/December of 2008 and 2009 for all 8 reaches in Kings Creek with standard error in parentheses (n = 3). Open canopy reaches (when open vs. closed were compared) had greater amounts of filamentous algae than closed canopy reaches (one-way ANOVA, p = 0.006).

	Canopy	Filamentous algae
Reach	%	g DM m^{-2}
NCU	66	0.37 (0.20)
NR-A	7	0.51 (0.29)
NCD	65	0.03 (0.03)
NO	7	8.54 (4.31)
KO	20	4.51 (0.76)
KCU	85	0.33 (0.21)
KR-A	4	5.74 (1.88)
KCD	80	1.72 (1.67)

Chapter 4 - Prairie stream responses to restoration through riparian woody vegetation removal

Abstract

Woody vegetation encroachment has become a major threat to remaining prairie streams, converting them from open to closed canopy. A century ago, Kings Creek, a prairie stream in northeast Kansas, was mostly bordered by native tallgrass prairie, but woody vegetation has spread along the stream channels. Stream reaches with a naturally open canopy were compared to reaches with a naturally closed canopy, and reaches where riparian woody vegetation was removed to assess the impact of woody encroachment on prairie stream structure and food web interactions. The effects of woody vegetation encroachment were studied as related to response to flooding. Wood and leaf standing stocks, filamentous algal biomass, chlorophyll *a*, were measured in the removal, naturally open, and naturally closed canopy reaches. Abundance of dead wood and leaves were greater with more canopy cover and chlorophyll and filamentous algae was less abundant. In 6 of 8 reaches, chlorophyll increased for 241 days post-flood and then declined. The filamentous algal biomass to chlorophyll ratio was greater in open-canopied reaches, demonstrating a shift in algal communities as a result of differences in canopy cover. In the vegetation removal reaches, shredders and FBOM in spring and summer became more depleted in $\delta^{13}C$ indicating a shift in the food web toward filamentous algae or deciduous tree leaves. It appeared that fish and crayfish diets were not impacted by canopy cover variation at the reach scale and these organisms were able to track food sources. Woody expansion impacts prairie stream structure as well as function and potentially alters resources available to the stream food web.

Introduction

Prairie stream ecosystem

Most ecosystems have been severely and directly altered by human activities (Vitousek 1994). Prairie streams are no exception, and they have become critically impacted by agriculture and urbanization. Over 95% of the once extensive North American tallgrass prairie has been destroyed (Samson and Knopf 1994). Most of the remaining prairie fragments are not large enough to encompass fully functional watersheds, which puts prairie stream habitats and organisms at risk (Dodds et al. 2004). Prairie streams and rivers are also home to threatened and endangered species, such as the Topeka shiner (*Notropis topeka*) and Neosho madtom (*Noturus placidus*).

Woody vegetation encroachment is one potential threat to the remaining fragments of tallgrass prairies. Decreases in fire frequency and intensity in the last century have allowed for increases in woody vegetation growth on prairies. One major route of establishment is along stream channels. An increase of shrubs and trees along riparian corridors converts open canopy prairie streams to closed canopy streams. Woody material along stream channels on Konza Prairie Biological Station (hereafter referred to as Konza) increased approximately 70% from 1939 to 2002 (Briggs et al. 2005). Thus, Konza can serve as a model for regional change as related to prairie streams.

Changes in canopy cover could alter prairie stream structure and function and could potentially make prairie streams more similar to forested streams. A decrease in sunlight could decrease the amount of primary production, and thus the amount of food available to grazers at the base of the stream food web. An increase in deciduous canopy cover could lead to a greater amount of coarse allochthonous input, and there could be seasonal patterns as a result of more

leaf input in the fall, providing more food to portions of the food web that rely on leaves, wood, and the microbes that degrade them. A change in prairie stream food web structure and the amount of allochthonous material could change carbon flux pathways and food web interactions.

A change in canopy cover could have several effects on stream structure that could impact higher trophic levels (e.g., invertebrates and fish). Many studies have investigated the effects of canopy cover on population dynamics and production in streams (e.g., Franken et al. 2007, Nystrom et al. 2003, Roy et al. 2005, Riley et al. 2009). It is important to understand how canopy cover can affect trophic structure; however, most studies investigate the effects of removing naturally occurring woody riparian canopy (e.g., alteration of the natural condition leads to less woody riparian cover). Comparing vegetation removal studies of naturally occurring woody riparian vegetation to studies where woody riparian vegetation is not natural can be difficult. Still, studies of the alteration of naturally occurring woody vegetation indicate basic changes in ecosystem structure and function with altered riparian canopies (e.g., Benstead et al. 2003, England and Rosemond 2004, Findlay et al. 1993). For example, Dineen et al. (2007) found that in Irish streams, trout density and biomass were generally greater in reaches that had a closed canopy (73-90% closed, the natural condition before humans deforested much of Ireland) when compared to grassland streams (0% closed canopy) or partially open canopied streams (13-54% closed). In contrast, small stream prairie fishes are adapted to open canopies and the intermittent flows that are common in prairie streams. If woody vegetation encroachment continues in prairie stream ecosystems, fish abundance could change as a result of shifts in food webs or other behavioral responses. An increase in the riparian canopy cover may allow for downstream species found in areas with closed canopies to move into these areas, and

this could have a negative impact on prairie-adapted fish. Little is known about the impacts of riparian change on food webs in these streams.

Riparian vegetation can impact food webs by altering terrestrial arthropod inputs, which can be a major food subsidy for some fish species (i.e., *Semotilus atromaculatus*, the creek chub, for the current study). A greater abundance of terrestrial arthropod input associated with a closed canopy system, can increase the abundance of predatory fish due to the availability of a high-quality food source (Nakano et al. 1999). The role of terrestrial arthropods was examined in a forested Japanese stream where terrestrial arthropod input was manipulated, and when inputs were decreased, the diet of fish shifted from terrestrial to aquatic arthropods (Nakano et al. 1999). Therefore, if an open canopy system converts to a closed canopy system, the riparian vegetation could directly impact food web interactions by altering the available food sources.

Objectives

The main goal for this study was to determine how woody vegetation encroachment affects prairie stream ecosystems and if structure and function could be restored to native conditions via removal of riparian woody vegetation at the reach-scale level. We had two specific questions: 1. Does canopy cover affect the amount and type of algal biomass in prairie stream reaches? 2. Does the diet of consumers change based on canopy cover?

First, we hypothesized that close canopied reaches would have a greater amount of leaf and wood material, and open canopied reaches would have more filamentous algal biomass as a direct result of more sunlight available for primary producers. Second, we hypothesized that consumer diets would shift to match available food sources. In other words, if more filamentous algae were present in open canopy reaches, consumers in those reaches would eat more filaments. If more leaves were present in closed canopy reaches, then more leaves (and not other

secondary food sources) would be consumed by leaf-eating consumers. We particularly expected to see such diet shifts in omnivorous animals.

Methods

Study site

Our study was conducted in Kings Creek during 2007-2009. Kings Creek is an intermittent prairie stream whose watershed is encompassed in native grassland within the Konza Prairie Biological Station. Konza Prairie, a tallgrass prairie preserve, is located in the northern part of the Flint Hills region near Manhattan, Kansas, and is owned by The Nature Conservancy and managed by the Division of Biology at Kansas State University. A detailed description of Konza and Kings Creek has been published previously (Gray et al. 1998; Gray and Dodds 1998).

The eight study reaches were located in two different subwatersheds on Konza (four reaches in each subwatershed). Subwatershed N04D is burned every four years and grazed by native American bison (*Bos bison*). N04D was burned in 2009 during the course of this study. Subwatershed K02A/AL (hereafter referred to as K02A) contained 1 reach in the top part of the study site that was not grazed and burned every two years (burned in 2008 during the course of this study). Three reaches at K02A, in the lower part of the study site, were located in an area that is not grazed by bison or cattle nor is it burned regularly (burned in 2009 during the course of this study). A detailed description of the riparian vegetation and reach characteristics (e.g., length, depth, width, velocity, and percent canopy cover) can be found in the previous chapter.

Each subwatershed consisted of four reaches that had differences in canopy cover either naturally, or related to experimental manipulation. For the purposes of this study, each reach was assigned a code that indicated the subwatershed and the type of riparian canopy cover. The first letter in the reach code represents the subwatershed, N for N04D and K for K02A. The

second letter represents the type of riparian canopy cover: O for naturally open canopy cover, C for closed canopy, and R for the vegetation removal reaches. A third letter was used for the naturally closed canopy reaches that were either upstream (U) or downstream (D) from the removal reach. At N04D, the order from upstream reach to downstream reach and the percent canopy cover was NCU (66%), NR (61% before and 7% after removal), NCD (65%), and NO (7%). At K02A, from upstream to downstream the reach order and percent canopy was KO (20%), KCU (85%), KR (83% before and 4% after removal), and KCD (80%). The removal reaches will be referred to as NR-B and KR-B for before removal results and NR-A and KR-A for after vegetation removal results.

Removal of the riparian vegetation occurred in December of 2007. The vegetation removal was maintained throughout the remainder of the study (2008 and 2009). Each fall before the deciduous trees dropped their leaves, a wire mesh fence (1 cm holes) was placed across the upstream side of the removal reach to catch leaves that washed downstream. Weekly, leaves were collected from the fence and moved into the downstream reach below the treatment. The fence was removed after leaf-fall and any leaves in the removal reaches were manually removed. Additional details of the woody vegetation removal are provided in the previous chapter.

Response variables

Response variables were measured three times each year when the greatest differences were expected due to seasonal changes in canopy cover. Sample collection occurred in the spring before the riparian deciduous trees had full leaf coverage, during the summer with full leaf coverage, and during the late fall to early winter after the leaves fell.

Chlorophyll *a* was measured three times a year for two years post vegetation removal (2008 and 2009). Sample collection occurred in April, July, and November/December and consisted of selecting five rocks from each reach attempting to avoid any sampling bias. Methods for measuring chlorophyll from whole rocks are described in detail in chapter 3 and were performed according to standard methods (APHA 1995; Sartory and Grobbelaar 1984).

Standing stock of coarse organic debris and filamentous algal biomass samples were collected during the same sampling times as when chlorophyll *a* was measured. Filamentous algae, dead wood, and leaves were collected within a 0.25 m^2 quadrat from five locations in each reach. Quadrats were tossed without bias and habitats were sampled approximately proportional to their occurrence in the reach (weighted by the estimate of pool and riffle surface area). An attempt was made to collect bryophytes for a biomass estimate but the presence of bryophytes within the quadrats was too infrequent. Filaments, wood, and leaves were manually gathered from each quadrat, separated, and dried at 60 ^oC for at least 24 hours. Dry mass was estimated for each reach by averaging mass per unit area in category from each of the 5 quadrats.

Suspended particulate organic matter (SPOM) and total suspended solids (TSS) were each measured three times a year during 2008 and 2009 (April, July, and November/December), for two years post vegetation removal. Each sampling event consisted of collecting one gallon of water from each reach during a time of baseflow when there was no obvious disturbance in the reaches. Water was collected within reaches from downstream to upstream in a manner that minimized re-suspension of benthic materials due to sampling. The water was stored under refrigeration and filtered in the laboratory within 24 hours. A known volume of water was filtered through pre-ashed (475 ^oC for 6 hours) and pre-weighed Whatman GF/F 24 mm filters (filters weighed on a Mettler AE 260 Deltarange balance). Filters were dried at 60 ^oC for a

minimum of 24 hours and re-weighed again for a dry mass. Then, filters were placed in a muffle furnace at 475 ^{o}C for 6 hours to burn off any organic material and weighed again. The difference between the dried filter and the pre-ashed filter gave TSS, and the difference between the dried filter and the ashed filter was used to calculate ash free dry mass per unit volume (SPOM). Analyses of suspended particulate material from Kings Creek demonstrated that re-wetting and drying were not necessary to obtain constant weight (data not shown). SPOM and TSS were measured in order to assess the difference in particle generation in the water column based on canopy cover.

Food sources (leaves, filamentous algae, bryophytes, fine benthic organic matter (FBOM), and epilithon), invertebrates, fish, and crayfish were collected during the spring and summer of 2007 (prior to vegetation removal) and 2009 (post vegetation removal) for food web characterization via analyses of natural abundance of δ^{13}C and δ^{15}N. In each reach, 10 leaves were collected, filamentous algae were gathered from 5 locations, and bryophytes were gathered from 5 locations. All samples from the same group were pooled together for one overall sample from each reach. FBOM was collected from each reach using suction to remove benthic detritus. Five rocks were randomly selected throughout each reach and a small brush was used to scrub epilithon from all surfaces of the rocks. Material was washed into the collection bag with de-ionized water. All food source samples were immediately frozen after collection.

Invertebrates were collected by flipping rocks, sorting through leaf packs, and using dip nets. Invertebrate prey were separated from predators and kept in containers for approximately 6 hours to allow for guts to empty. Invertebrates were then identified to family and frozen until further processing. Fish and crayfish were collected using a backpack electroshocker. Fish species collected included *Etheostoma spectabile* (orange throat darter), *Campostoma anomalum*

(central stoneroller), *Semotilus atromaculatus* (creek chub), and *Phoxinus erythrogaster* (southern redbelly dace). Crayfish species collected were *Orconectes neglectus* and *Orconectes nais*. Length of each animal was measured, and then they were frozen until tissue processing. For fish > 25mm a muscle tissue sample was taken. If the fish was < 25 mm, the gut contents were removed and the whole body was used. Muscle tissue was removed from crayfish tails for isotopic analysis. All samples were dried in a Labconco freeze-dryer and placed in a dessicator until they were ground. All samples were ground with a mortar and pestle, except for leaves, which were ground with a coffee grinder.

The limestone bedrock in Kings Creek is a source of calcium carbonate, and precipitation from streamwater on solid materials can influence the $\delta^{13}C$ value of samples. Stable isotope samples that were potentially influenced by calcium carbonate accumulation were weighed into silver capsules, and acidified with 50 µL of 0.1 M HCl. Once the sample stopped bubbling, the sample was treated with 50 µL of 0.1 M NaOH to neutralize the acid. The acidified samples were dried overnight at 60 ^{o}C before the capsules were closed. Acidified samples were only analyzed for $\delta^{13}C$. A separate, unacidified sample was weighed, packed into tin capsules and analyzed for $\delta^{15}N$. Samples that did not have a buildup of calcium carbonate (e.g., fish and crayfish muscle material) were weighed and packed into tin capsules, and were analyzed for $\delta^{13}C$ and $\delta^{15}N$ from the same sample. All food sources (leaves, filamentous algae, bryophytes, FBOM, and epilithon) were acidified.

Invertebrate samples were grouped by the following functional feeding groups: gatherer, scraper, shredder, filterer, or predator. Gatherers were not present frequently enough to be included in analyses. If the functional feeding group contained a family that commonly had visible calcium carbonate accumulation when viewed microscopically, then the sample was

acidified. Samples were sent to the Stable Isotope Mass Spectrometry Laboratory in the Division of Biology at Kansas State University where a ThermoFinnigan Delta Plus mass spectrometer was used for isotopic analysis. Standards were analyzed at least every 12 samples; the standard deviation range for ^{15}N was 0.03 to 0.18‰, and the range for ^{13}C was 0.02 to 0.09‰.

The difference in $\delta^{13}C$ and $\delta^{15}N$ before to after the removal was calculated to indicate shifts in consumer food sources in the removal reaches. Samples were compared from spring and summer in 2007 (before the removal) and spring and summer in 2009 (one year after the removal). For example, the $\delta^{13}C$ value for shredders in spring 2009 was subtracted from the spring 2007 value. This calculation was done for each invertebrate functional feeding group, fish, and crayfish for each reach. The difference between summer 2007 and 2009 values and the difference in $\delta^{15}N$ were calculated.

Canopy cover and days since flood were measured as independent variables influencing the food web compartments. We controlled for days since flood to separate out the effects of canopy removal and were interested in how canopy influenced trajectory of recovery of primary producers. The number of days that had passed since the previous flood was used as an independent variable in determining flood effects on the response variables measured. Floods were determined using 30 years of discharge data from the U.S. Geological Survey gauging station (# 06879650) immediately downstream as those events that exceeded a 1.67 annual return interval (Fritz and Dodds 2005). Presence or absence of canopy visible in a densiometer was used to calculate average percent canopy cover for each reach. Details on the methods for determining the number of days since flood and percent canopy are described in the previous chapter.

Statistical analysis

All statistical analyses were performed using the program Statistica (version 6.1, StatSoft Inc., Tulsa OK, USA). Initial exploration of data was done with the nonparametric Kendall Tau correlation analysis on response variables (filamentous algae biomass, chlorophyll *a*, filamentous biomass:chlorophyll *a* ratio, SPOM, and TSS). Wood and leaf standing stocks were not analyzed with correlation because these materials were kept out of the removal reaches with a mesh fence in the fall, and materials that did enter the removal reaches were manually removed after all leaves fell from the riparian trees. Significant Kendall Tau correlations were used to determine what analysis of covariance (ANCOVA) tests to run (e.g., to remove highly cross correlated independent variables). ANCOVA was used to simultaneously assess categorical (e.g., season, watershed, open/closed) and continuous (days since flood and percentage canopy cover) variables. Chlorophyll *a*, filamentous biomass:chlorophyll *a* ratio, filamentous algal biomass, and wood and leaf standing stock data were from 2008-2009 (i.e. only post removal data). SPOM and TSS data were also from 2008-2009 and the ANCOVA tests on SPOM and TSS data were analyzed with the reaches categorized as open or closed canopy. Reaches were determined to be open or closed based on the reach code, and it was not based on seasonal changes in canopy cover. Therefore, NR, NO, KR, and KO were open canopy reaches. NCU, NCD, KCU, and KCD were closed canopy reaches. SPOM and TSS were not significantly different between open and closed canopy reaches so those data are not presented.

Results

Standing stock, biomass, and chlorophyll

The standing stock of wood was combined by year and season to determine the effect of canopy cover (Fig. 4.1). The amount of wood present in a reach increased with percentage

canopy cover. Reach KR-A had the lowest percent canopy (4%), and the amount of wood was 5.7 times greater in reach KCU, which had the greatest percent canopy cover (85%). For the amount of wood, the removal reaches were more similar to the naturally open reaches than the closed canopy reaches.

The standing stock of leaf material was significantly different among reach categories. Leaves were positively related to canopy cover indicating that the amount of leaves present in a reach increased with percentage canopy cover (Table 4.1). The positive relationship with canopy cover and leaves was only evident in the fall (Fig. 4.1). During spring, the average amount of leaf material in open canopy reaches was 34 g DM m^{-2}, and the average for closed canopy reaches was 36 g DM m^{-2}. Summer averages were also similar for open and closed canopy reaches, with 1.7 and 2.1 g DM m^{-2}, respectively. The similarity in the amount of leaf material between open and closed canopy reaches during spring and summer would be the result from decomposition, floods washing leaves away, and limited inputs this time of year. During fall, open canopy reaches had an average leaf weight of 41 g DM m^{-2}, and closed canopy reaches had an average of 310 g DM m^{-2}. Reach NO had the least amount of leaf material, and KCD had the most with 17 times more than NO. Therefore, the removal reaches mimicked the naturally open reaches in the fall, but naturally and experimentally open and closed reaches all have a similar amount of leaves in spring and summer.

Filamentous algae biomass was negatively correlated with greater canopy cover (Table 4.2). ANCOVA results for canopy cover when filaments were the dependent variable were also significant (Table 4.3). Filament biomass was separated by season to determine the seasonal impact of canopy cover. Generally, open reaches may or may not have filaments, but closed canopy reaches always had very low filament biomass (Fig. 4.2). Filament biomass as a function

of percentage canopy cover showed that the canopy affect was strongest in the fall with an open canopy reach (NO) having more than 100 times the dry mass of filaments per unit area as a closed canopy reach (NCD), coinciding with maximum leaf standing stocks. There was no relationship between filament biomass and canopy in the summer because filaments were only found in two reaches. However, the two reaches where filaments were found were open (KR-A and NO). The spring relationship was weaker than the fall because it was mostly driven by filaments found in NO.

Correlation analysis also revealed that the number of days since the last flood was correlated with filament biomass (Table 4.4). Some sampling events in the two watersheds occurred on slightly different days, so results were separated by watershed (Fig. 4.3). Following a flood, it appeared that there was a pulse in filament growth in open canopy reaches that was not evident in closed canopy reaches. The slight effect of days since flood on filament length in open canopy reaches was supported by a significant result (Table 4.3).

Correlation analysis showed that chlorophyll *a* data were not significant with percent canopy cover across all sampling locations and seasons (Table 4.2). However, simultaneously assessing categorical and continuous variables showed that canopy cover was significantly negatively related to chlorophyll *a* (Table 4.5). When chlorophyll values were combined by season and separated by year, there was no strong pattern with canopy cover (Fig. 4.4). In 2008 and 2009 NCD had the lowest chlorophyll concentration and KR-A had the greatest, with 2.2 and 2.7 times more, respectively. Day since flood was significant with chlorophyll *a* (Table 4.5), and they were marginally correlated (Table 4.4). In 6 of 8 reaches, chlorophyll *a* increased up until 244 days since flood and then decreased (Fig. 4.3). NCD and KCU increased in chlorophyll *a* concentration up to 145 days post flood and then decreased.

After the removal it was visually obvious that the removal reaches had more filamentous algae than the closed canopy reaches. Therefore, the ratio of filamentous algal biomass to chlorophyll *a* concentration was calculated to indicate shifts in algal community structure. The ratio decreased with an increase in canopy cover indicating filaments composed more of the total algal biomass in open reaches (Fig. 4.5). Canopy cover and the filament:chlorophyll ratio were significantly correlated (Table 4.2). ANCOVA results showed that canopy was significant when the ratio was the dependent variable (Table 4.6). Reach NO had the greatest ratio which was more than 130 times greater than the lowest ratio in NCD.

Natural Abundance of Stable isotopes

The range in $\delta^{13}C$ and $\delta^{15}N$ values for food sources showed that there was substantial overlap in isotopic signatures (Table 4.7). The overlap in the range of food sources makes it difficult to determine the difference between consumers eating leaf-based diets (most likely associated with closed canopy) versus filamentous algae-based diets (most likely associated with open canopy). The averages across all reaches were different and revealed that leaves, algae, and bryophytes were more depleted in ^{13}C and ^{15}N than epilithon and FBOM samples (Fig. 4.6).

Biplots of $\delta^{13}C$ and $\delta^{15}N$ for invertebrate functional feeding groups revealed that reaches did not group together based on canopy cover, but grouped by watershed, season, or year (Fig. 4.7). Filterers grouped by watershed, with N04D being more enriched in ^{15}N than K02A. N04D filterers were more enriched in ^{13}C than K02A spring and summer 2009 filterers.

Generally scrapers grouped by watershed, season, and year. K02A spring 2007 scrapers were the most depleted in ^{13}C, and summer 2007 scrapers from both watersheds were the most enriched in ^{13}C. K02A spring and summer 2007, and summer 2009 scrapers were the most depleted in ^{15}N. N04D spring 2007 and summer 2009 scrapers were the most enriched in ^{15}N.

K02A spring and summer 2009 scrapers were more depleted in ^{15}N than N04D spring and summer 2009 scrapers.

Shredders grouped by season, however there were fewer summer samples than spring samples. The summer shredder samples were the most enriched in ^{13}C. Shredder samples from NR-A and NO in spring 2009 grouped next to each other, as did KR-A and KO spring 2009.

Invertebrate predators grouped by year and season. Spring 2007 predators from both watersheds were tightly grouped together and were the most depleted in ^{13}C. Summer 2007 predators from both watersheds grouped together. Predators from 2009 overlapped with the 2007 predators in ^{15}N, but 2009 predators had a greater range and were more enriched in ^{15}N.

The isotopic signatures of fish and crayfish species collected in August 2009 were compared to each other. A biplot of $\delta^{13}C$ and $\delta^{15}N$ values from four fish species and crayfish showed that samples grouped by species with some overlap in isotopic signatures (Fig. 4.8). *E. spectabile* were the most enriched in ^{15}N. *C. anomalum* from K02A were the most depleted in ^{13}C, and individuals from N04D overlapped with other species. *S. atromaculatus* were the most enriched in ^{13}C, most likely due to a diet of terrestrial insects. *P. erythrogaster* had isotopic signatures that overlapped with other species. Dace were most similar to darters, creek chub and stonerollers from N04D in ^{15}N. Dace were most similar to darters, crayfish, and stonerollers from N04D in ^{13}C. Crayfish were the most depleted in ^{15}N.

The difference in $\delta^{13}C$ for isotope samples from 2007 and 2009 for spring and summer was calculated to determine how food web interactions were shifting in the vegetation removal reaches before and after treatment. There were two invertebrate functional feeding groups where differences consistent with trends related to canopy manipulation (Fig. 4.9). A positive difference means the source became more depleted in ^{13}C and indicates shift toward filamentous

algae or deciduous leaves as potential food sources. Filamentous algae as a food source would most likely be associated with an open canopy, and leaves as a food source would most likely be associated with a closed canopy, particularly in the fall. A negative difference means the source became more enriched in ^{13}C. A sample more enriched in ^{13}C indicates a shift away from filaments and leaves and towards epilithon or FBOM as potential food sources.

In the removal reaches during spring, shredders shifted more positively (towards filaments or leaves) than the other reaches, with KR shifting 3.1 times more than NR. Also during spring, all reaches had a negative difference (shift towards epilithon or FBOM) for darters and the removal reaches were more negative than the other reaches. NR was 1.8 times more negative than KR. Due to overlap in $\delta^{13}C$ values for food sources, it is difficult to determine the exact food source a functional feeding group shifted towards.

In the difference in isotopic signature plots, similar to ^{13}C, a positive difference in ^{15}N indicates a shift to a more depleted ^{15}N source, such as filamentous algae or deciduous leaves. A negative difference in ^{15}N indicates a shift to a more enriched ^{15}N source, which is more similar to epilithon or FBOM. The difference in $\delta^{15}N$ had notable positive or negative shifts in three invertebrate functional feeding groups and crayfish (Fig. 4.10). Shredders in the removal reaches during spring had a negative difference between 2007 and 2009. Scrapers in the spring had a negative difference in KR and a positive difference in NR. All reaches in K02A had a negative difference, and all reaches in N04D had a positive difference. Predators in the summer had a negative difference in the removal reaches. NCD was the only reach that had a positive difference. During the summer, crayfish in KR had a positive difference, and crayfish in NR had a negative difference, indicating no consistent detectable difference in food sources for the

omnivores. Additional results of the difference in isotopic signatures for consumers from before to after the removal are provided in the appendix.

Discussion

Most published studies on alterations of riparian canopy evaluate transitions from open to closed canopy; however the natural condition of the low order, headwater reaches of Kings Creek is open canopy, as it is surrounded by tallgrass prairie. Numerous studies comparing shaded streams to open canopy streams deal with deforestation (i.e., Banks et al. 2007; Kiffney and Bull 2000; Melody and Richardson 2007). It is difficult to compare the effects of woody vegetation removal from this study to other studies because clearcutting impacts the surrounding land, as heavy equipment is required, and bare soil can generate substantially more sediment load than soil covered by natural grasslands. For example, a headwater stream in Canada had greater amounts of inorganic matter (mostly fine sediment) in logged streams (open canopy) than forested areas (Kiffney and Bull 2000), a result of bare soil from the disturbance of logging, whereas we saw no significant difference in particulate materials.

Standing stock, biomass, and chlorophyll

The standing stocks of wood and leaves were measured to determine how much material was present in open canopy versus closed canopy reaches, and to be certain the treatment effect of canopy removal was successful. Overall, wood material did increase with canopy cover. As would be expected from other studies (and common sense), wood does increase with canopy cover (Roy et al. 2005).

The amount of leaves in the stream had a strong seasonal pattern. During spring and summer there was not an increase in leaf material as canopy increased, partially due to decomposition of leaves and the fact that leaf input is limited during the spring and summer.

Additionally, most floods in Kings Creek occur in late spring and early summer. Flooding would wash leaves away and decrease the local (reach-scale) effects of canopy cover on the standing stock of leaves. The strong relationship between the amount of leaves and canopy cover for naturally open and closed canopy reaches in the fall indicates that canopy does affect standing stocks of leaves in the stream channel at the reach scale. The wood and leaf results showed that closed canopy reaches had greater standing stocks than naturally open reaches. The results also demonstrated that the treatment of canopy removal was successful in making the removal reaches closer to naturally open canopy reaches with respect to standing stock of organic materials.

For this study, the number of days since flood influenced food web characteristics, as would be expected from other research on prairie streams (Dodds et al. 1996, Bertrand et al. 2009, Murdock et al. 2011). Floods had a more pronounced impact on chlorophyll *a* concentrations than on filamentous algal biomass, probably because filaments senesce after a period of time. Filaments often appear to be overgrown by epiphytes, but also may be more susceptible to a developing grazer community (Murdock et al. 2010). These additional processes make it more difficult to determine the long term impact of floods on filaments. It appeared that floods had some impact on the filaments in open canopy reaches, which could be because there were less filamentous algae present in closed canopy reaches to be affected by floods.

Filamentous algal biomass and chlorophyll *a* decreased with an increase in canopy cover, suggesting that woody vegetation encroachment could impact this basal food source. The idea that canopy influences algal communities is supported by other studies. Forested African streams had greater algal species richness than deforested open canopy reaches (Bixby et al. 2009). The study of a stream in Spain found similar results, where algal biomass and the

percentage of stream surface covered with algae were greater in logged areas than closed canopy areas (Sabater et al. 2000). Therefore, our results support these patterns that closing in of canopy cover over prairie streams can alter algal community structure as indicated by the relative dominance of filamentous algae.

The filamentous algal biomass:chlorophyll *a* ratio indicates a structural change occurring in algal composition, with more filamentous algae in open canopied reaches. Open canopy was the condition of Kings Creek when Europeans settled the area, and an increase in canopy cover could affect food web interactions. The abundance of grazers or herbivorous fish could decrease as the canopy closes in, due to a shift in food sources from filamentous algae to leaf material.

Food web interactions

Stable isotope analysis allowed for the examination of food web interactions in open and closed canopy reaches to determine if consumer organisms were shifting toward leaf-based diets associated with closed canopies or if there was a shift toward algal-based diets associated with open canopies. The high degree of overlap in ^{13}C values for leaves and filamentous algae made it difficult to determine distinct shifts in the food web related to canopy cover. The overlap in food source isotopic signatures also made it difficult to determine the difference between a shift towards epilithon or FBOM as a food source.

The current study did not include quantitative invertebrate sampling. However, canopy cover is not related to invertebrate abundance in some studies, but it is in others. The study of an Oregon desert stream (i.e. open canopy) found that collector, shredder, and predator biomass and abundance of all invertebrate groups did not change with canopy density (Tait et al. 1994). A study in Canada found that grazer biomass was not significant with canopy cover, but was significant with phosphorous concentration (Bourassa and Cattaneo 1998). The significance of

phosphorous suggests that human-caused eutrophication may have bigger influences than alterations in degree of canopy cover. A study in Australia determined that total invertebrate biomass did not differ between a forest and pasture stream (i.e. open canopy), however forest sites had greater shredder biomass and lower grazer biomass (Reed et al. 1994), suggesting that canopy may not equally affect all invertebrate functional feeding groups. In the current study, there were some shifts in ^{13}C and ^{15}N in the vegetation removal reaches, but the relationships were complex. Given overlapping food sources, we could not assess some potential changes in the food web that we would predict given differing abundance of leaves and filamentous algae in open and closed reaches. Riparian vegetation can also influence the abundance of terrestrial insects (Edwards and Huryn 1996), which are a food source for some fish species (terrestrial insects were not collected in the current study).

Even though it was difficult to detect specific shifts in food web interactions related to canopy cover, the ^{13}C and ^{15}N values indicate that some invertebrate functional feeding groups did have signatures related to previous food web linkages determined for Kings Creek (Stagliano and Whiles 2002). Based on invertebrate consumption estimates, Stagliano and Whiles (2002) determined that shredders consumed mostly CPOM (the majority of CPOM was leaf material), filterers consumed mostly SPOM, scrapers consumed mostly primary producers, and predators consumed mostly other invertebrates. In our study, the ^{15}N values for scrapers were lower than ^{15}N for filterers and predators, indicating that scrapers were closer to filamentous algae in ^{15}N. Shredders had similar ^{13}C and ^{15}N values as leaves, supporting the conclusion that shredders consumed mostly CPOM containing leaf material. Predators had ^{15}N values greater than signatures from food sources and other invertebrate groups, demonstrating that predators do prey on other invertebrates.

In addition to invertebrate functional feeding groups, the isotopic signatures of fish and crayfish also gave insight into food web interactions in Kings Creek. Fish and crayfish probably move greater distances within the stream than other organisms and stable isotope analysis indicated that canopy cover did not affect the diet of fish and crayfish. Analysis of fish and crayfish from summer two years after the vegetation removal indicated some overlap in the isotopic signature of some species, which could result from an overlap in diet. *E. spectabile* eats invertebrates. Generally, *P. erythrogaster* are herbivores; however it is possible that younger fish eat invertebrates. The degree of overlap in signatures suggests that during summer, the *P. erythrogaster* community consisted of younger individuals that were eating invertebrates. The presence of younger individuals in summer is supported by observations made during previous sampling (personal communication Erika Martin).

C. anomalum is an omnivore and individuals from K02A were more herbivorous and did not appear to be eating invertebrates, whereas for N04D it appeared that algae and leaf material was less of an important food source. This is consistent with lower standing stocks of filamentous algae in N04D. In contrast to what the isotopic signatures indicate, the analysis of gut contents revealed that *P. erythrogaster* and *C. anomalum* mainly consisted of algae and detritus regardless of canopy type (personal communication Kirk Mammoliti).

S. atromaculatus typically eat terrestrial items that fall into the stream. Larger individuals of *S. atromaculatus* may be piscivorous, but that did not appear to be the case in Kings Creek. Gut content analysis revealed that diet of *S. atromaculatus* consisted mostly of terrestrial and benthic invertebrates (personal communication Kirk Mammoliti).

Crayfish diet can vary widely depending on habitat. Sometimes crayfish eat detritus, they can be omnivorous, or they can be predators. During the summer, vascular plant detritus

could also be a main food source for *Orconectes* spp. based on previous gut content analysis (Evans-White et al. 2003). For the current study *Orconectes* spp. from open and closed canopy reaches were herbivorous during summer, indicating that algae or leaves were the main food source, and other food sources were not important. The gut contents from the crayfish in the current study were mainly algae and detritus, regardless of canopy type (personal communication Kirk Mammoliti). A crayfish diet of mostly algae is consistent with a previous study of crayfish in Kings Creek (Evans-White et al. 2001). The importance of algae in crayfish diet suggests that crayfish were able to switch food sources with changes in canopy cover that were associated with the removal of riparian woody vegetation. Crayfish tracking a food source indicates that mobile organisms can track food sources at scales greater than reaches.

Conclusions

The overall main goal was to determine how woody vegetation encroachment occurring on tallgrass prairie impacted the stream, and if removal of woody vegetation had significant effects on ecosystem structure and function at the reach scale. Woody vegetation encroachment changes prairie stream structure via greater standing stocks of leaf and wood material in closed canopy reaches and a shift in algal composition towards filaments in open canopy reaches. Stable isotope analysis revealed that food web interactions for invertebrates, fish, and crayfish were not strongly influenced by the type of canopy cover. Larger organisms (i.e. fish and crayfish) had a tighter range in ^{13}C and ^{15}N values than invertebrate groups perhaps because fish and crayfish can move greater distances and find specific food sources. Therefore, it is possible that riparian vegetation encroachment could have more of an impact on smaller organisms.

Tallgrass prairies historically had very little riparian woody vegetation, and if the current trends continue, prairie streams will eventually become forested streams. The current study

demonstrated that removal of riparian vegetation at the reach scale did restore natural conditions to some degree. It is possible that larger-scale woody vegetation removal experiments would have stronger influences on animal communities. Ideally, prairie streams would be preserved before woody vegetation encroachment became a problem, but restoration may be a possibility for land managers wanting to conserve the native state of prairie streams.

Figure 4.1 (A) Mass of wood in stream channel as a function of percentage canopy cover. (B) Mass of leaves in stream channel during the fall (after leaves fell) as a function of percentage canopy cover (ANCOVA, p = 0.002).

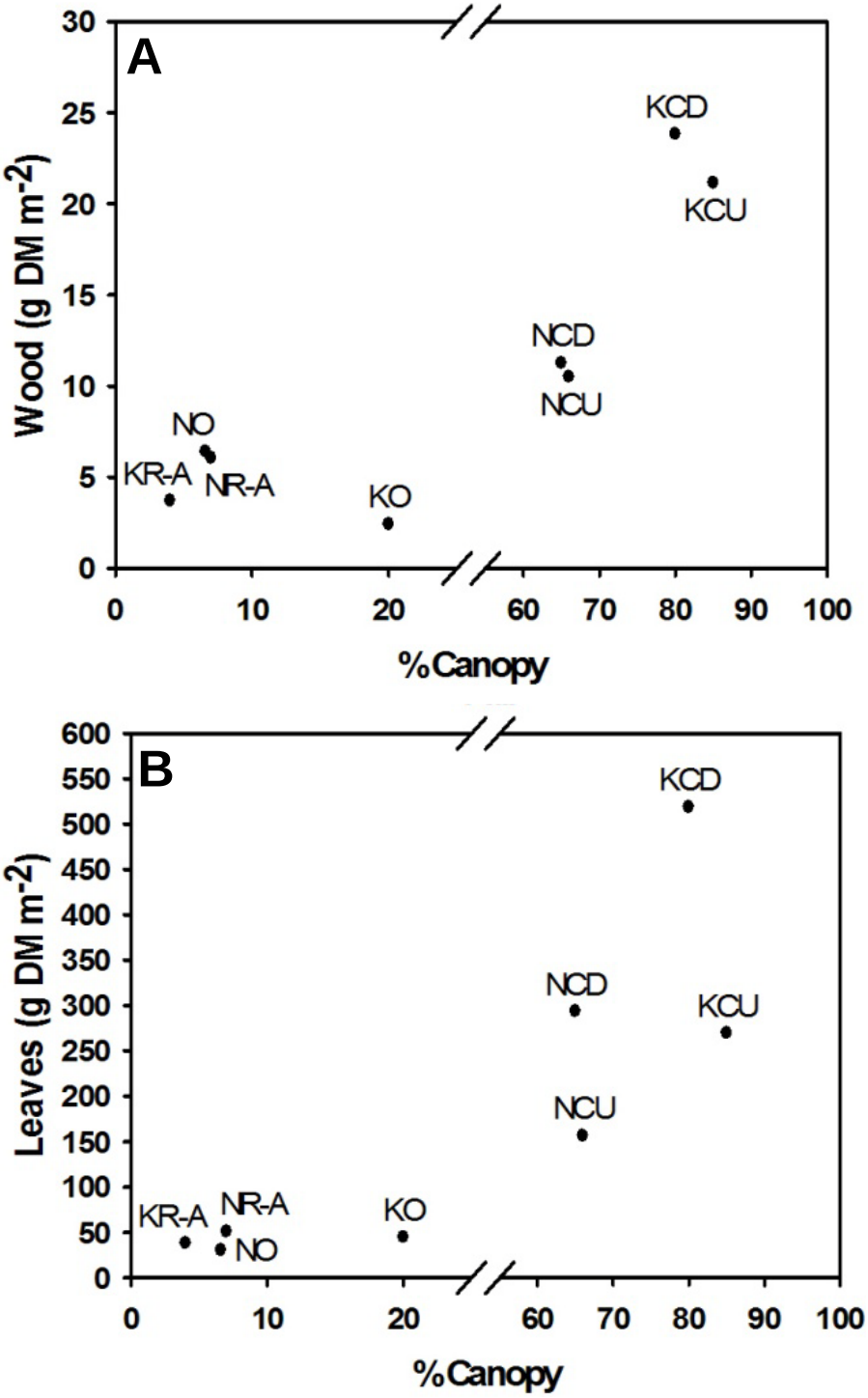

Figure 4.2 Patterns of filamentous algal biomass in the spring (A), summer (B), and fall (C) as a function of canopy cover (Kendall Tau, p = 0.015; ANCOVA, p = 0.008).

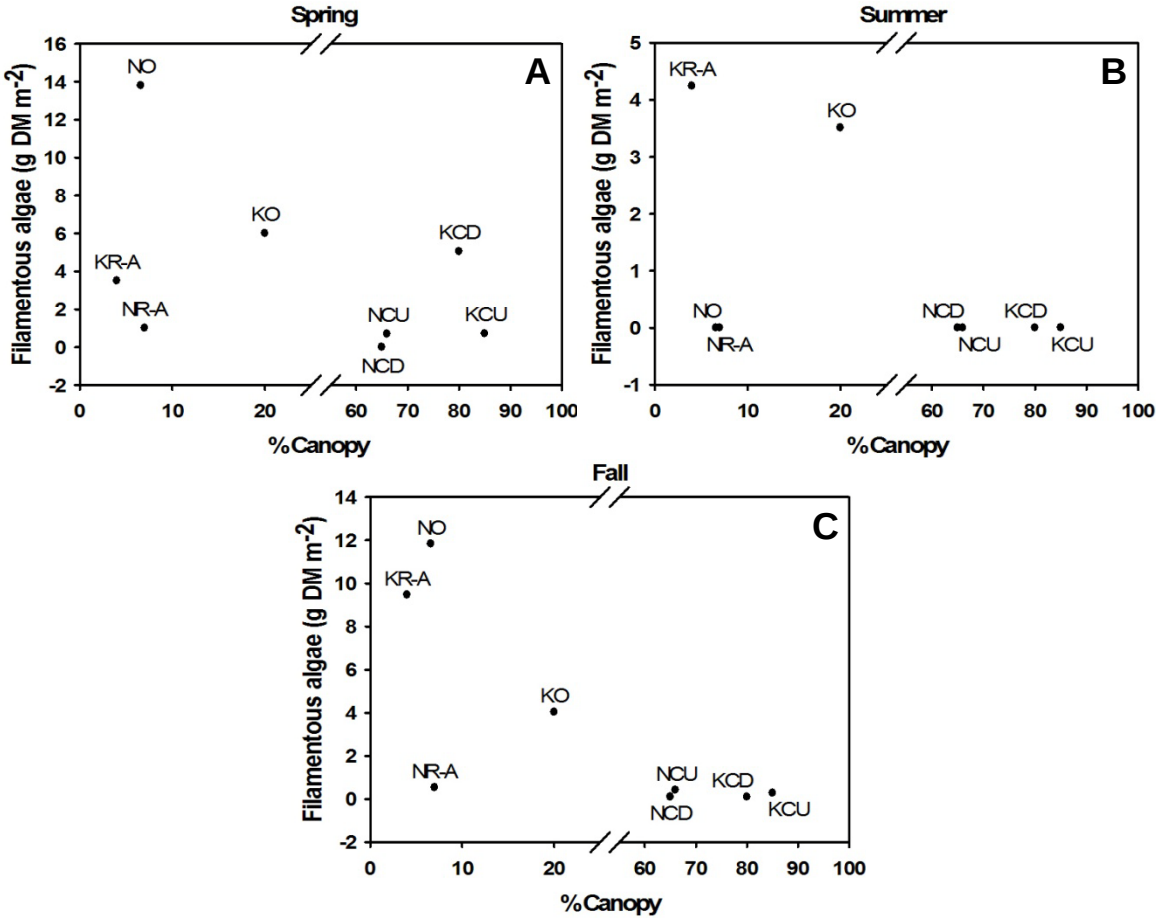

Figure 4.3 Filamentous algal biomass in N04D (A) and K02A (B) as a function of days since flood (ANCOVA, p = 0.04). Chlorophyll *a* concentration in N04D (C) and K02A (D) as a function of days since flood (ANCOVA, p = < 0.001).

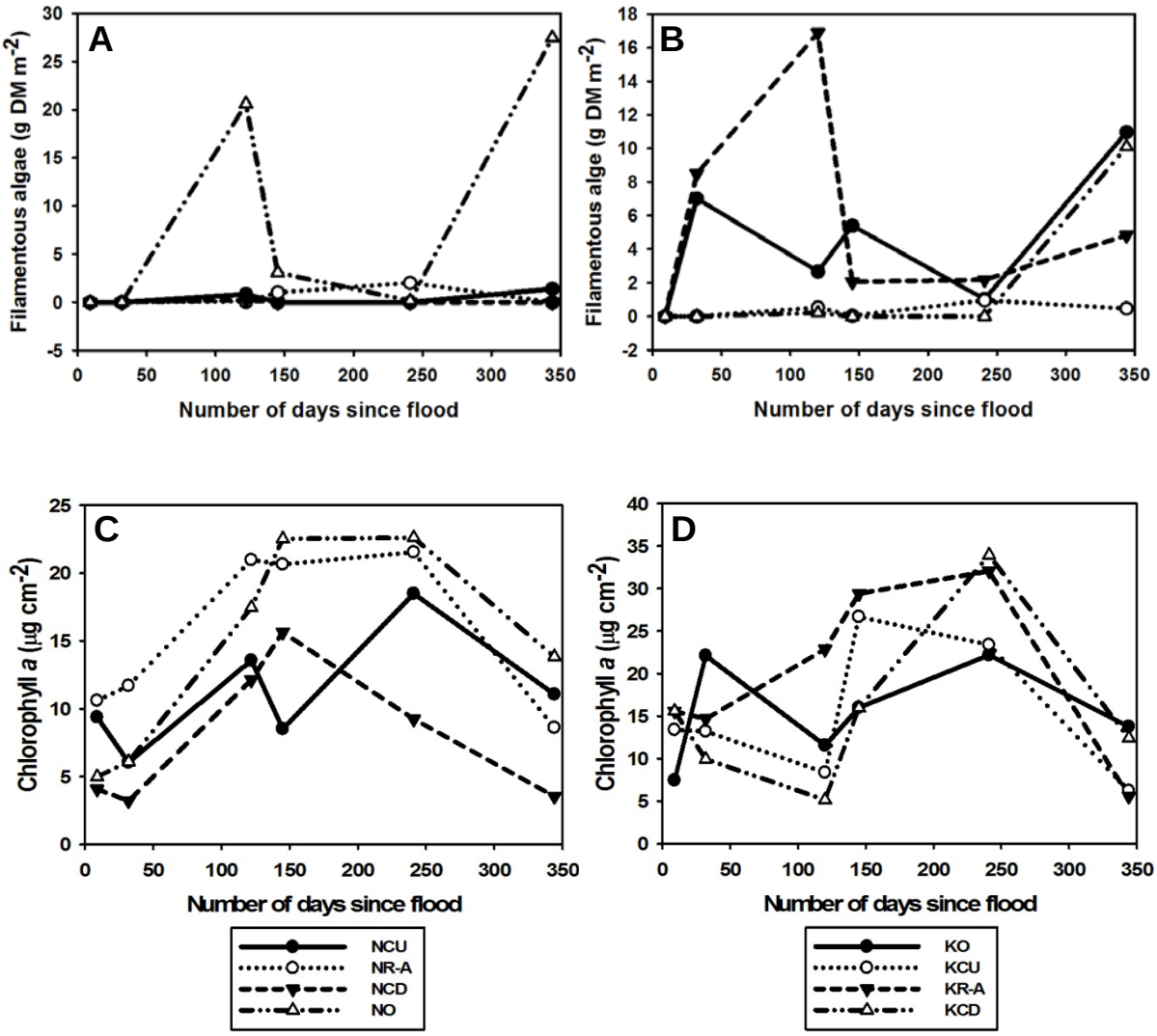

Figure 4.4 Chlorophyll *a* concentrations from 2008 (A) and 2009 (B) as a function of percentage canopy cover (ANCOVA, p = 0.019).

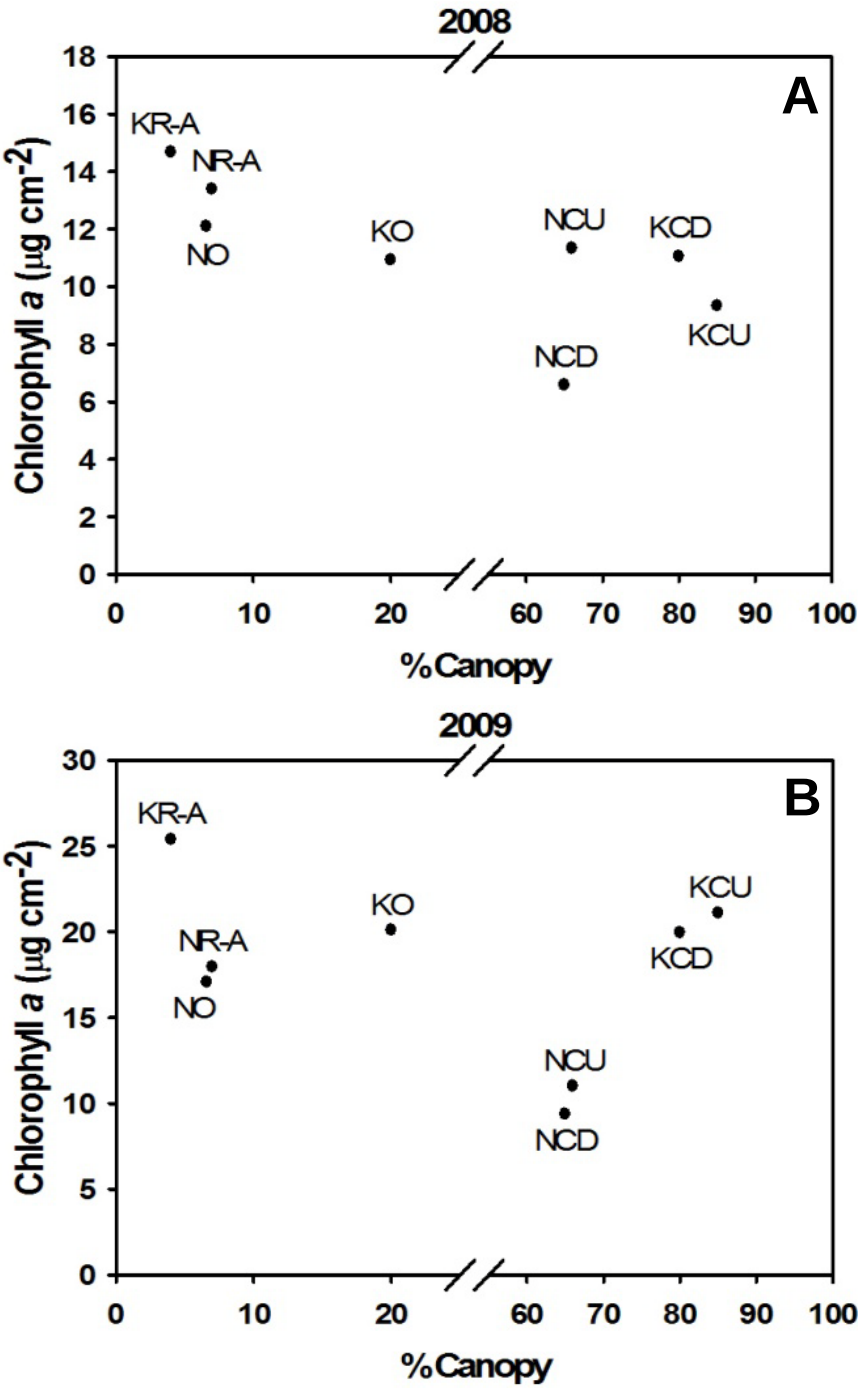

Figure 4.5 Filamentous algal biomass to chlorophyll *a* concentration ratio as a function of percentage canopy cover (Kendall Tau, p = 0.032; ANCOVA, p = 0.009).

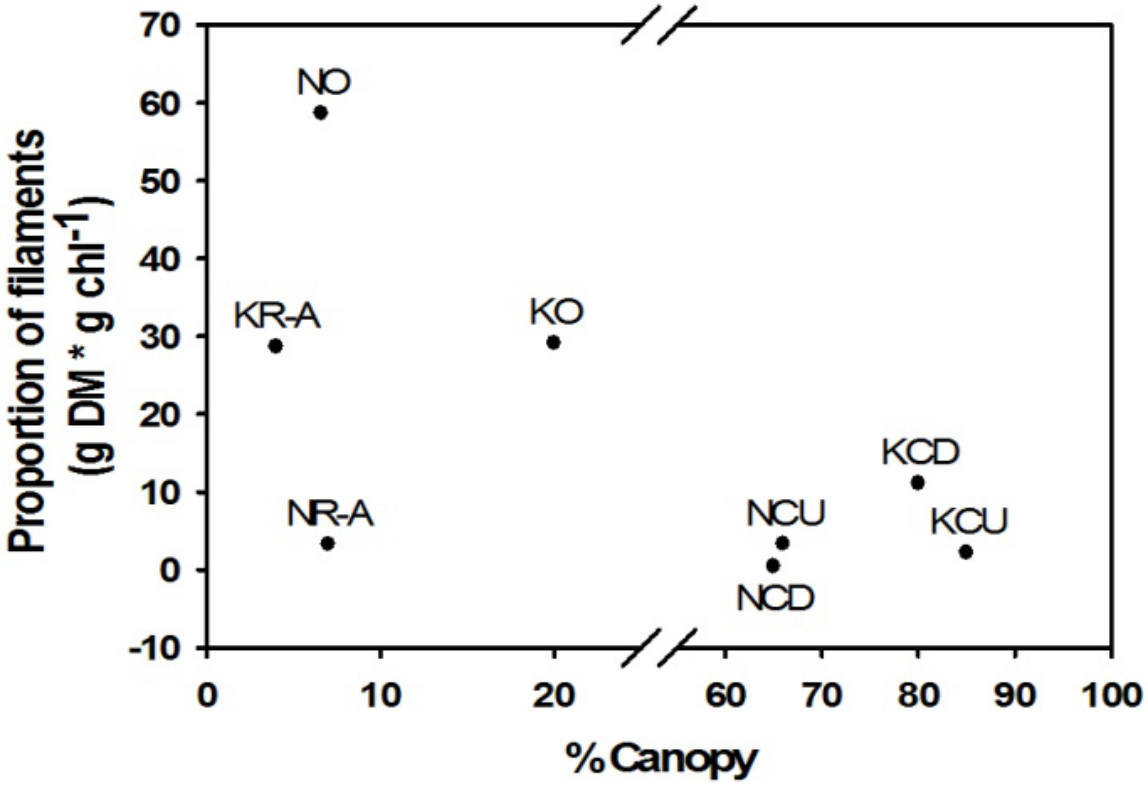

Figure 4.6 Average $\delta^{13}C$ and $\delta^{15}N$ values of food sources collected during spring and summer 2007 and 2009. The abbreviation for filamentous is 'Fil.' Error bars represent standard error.

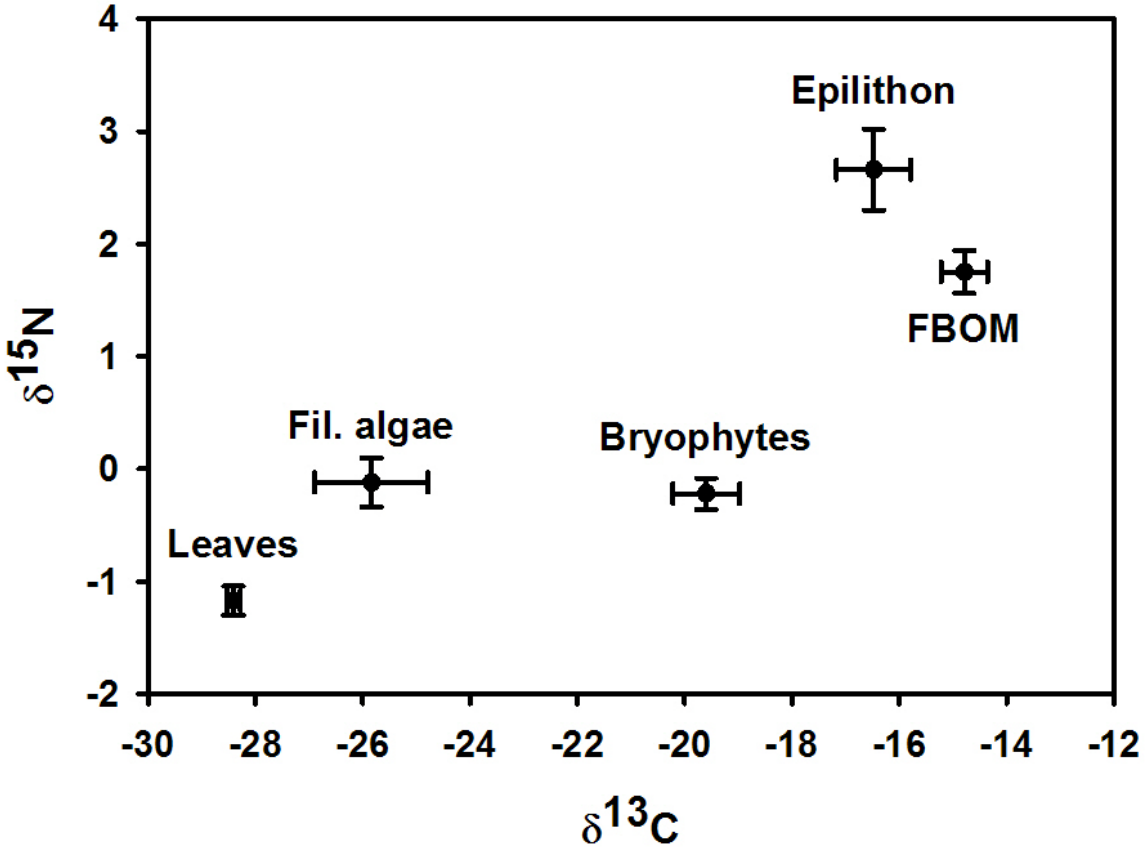

Figure 4.7 Average $\delta^{13}C$ and $\delta^{15}N$ values for invertebrate functional feeding groups: filterers (A), scrapers (B), shredders (C), and predators (D). Samples were collected during spring and summer 2007 and 2009.

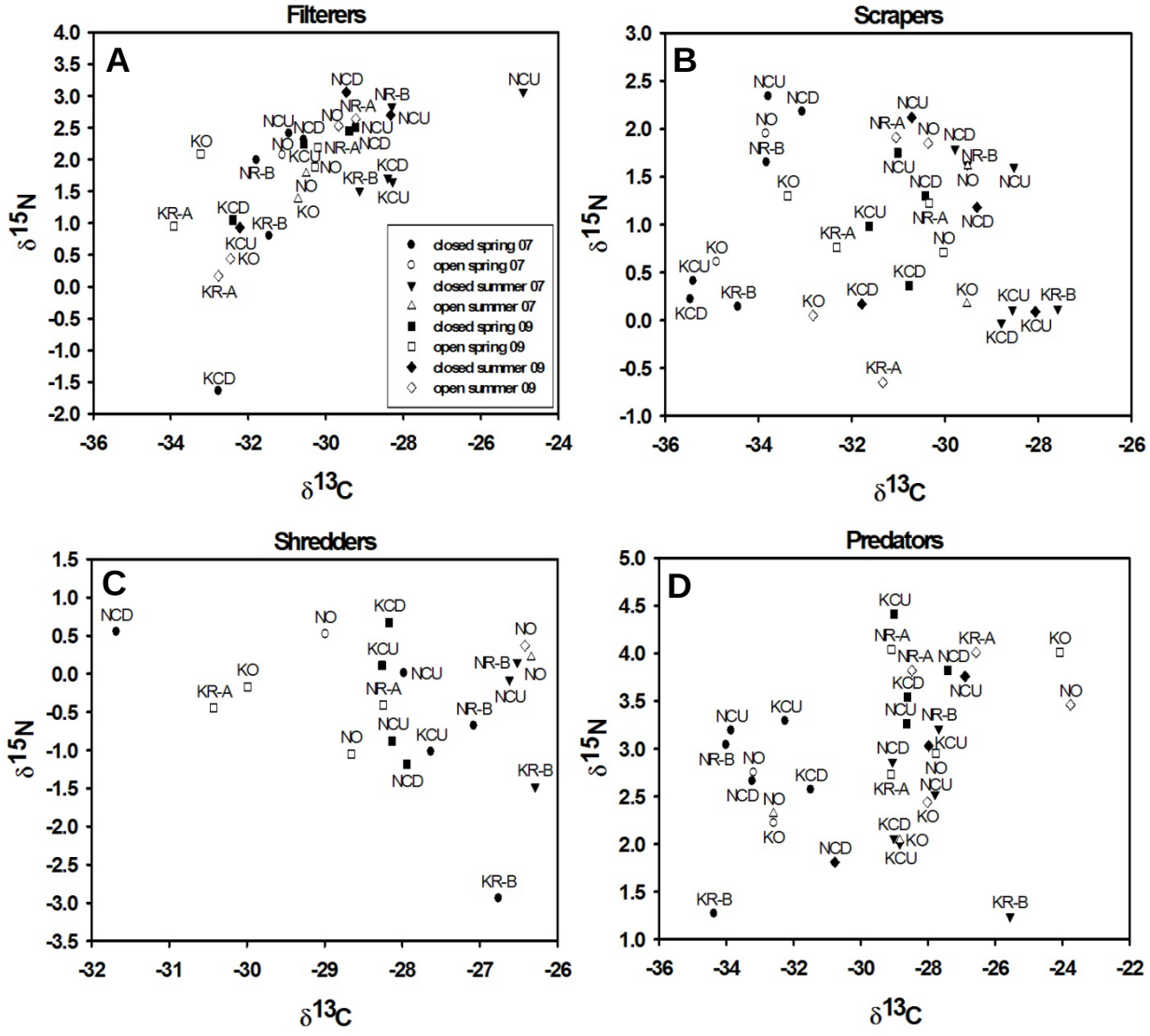

Figure 4.8 Average $\delta^{13}C$ and $\delta^{15}N$ values for fish and crayfish collected during August 2009: *Etheostoma spectabile* (A), *Campostoma anomalum* (B), *Semotilus atromaculatus* (C), *Phoxinus erythrogaster* (D), and *Ocronectes* spp. (E).

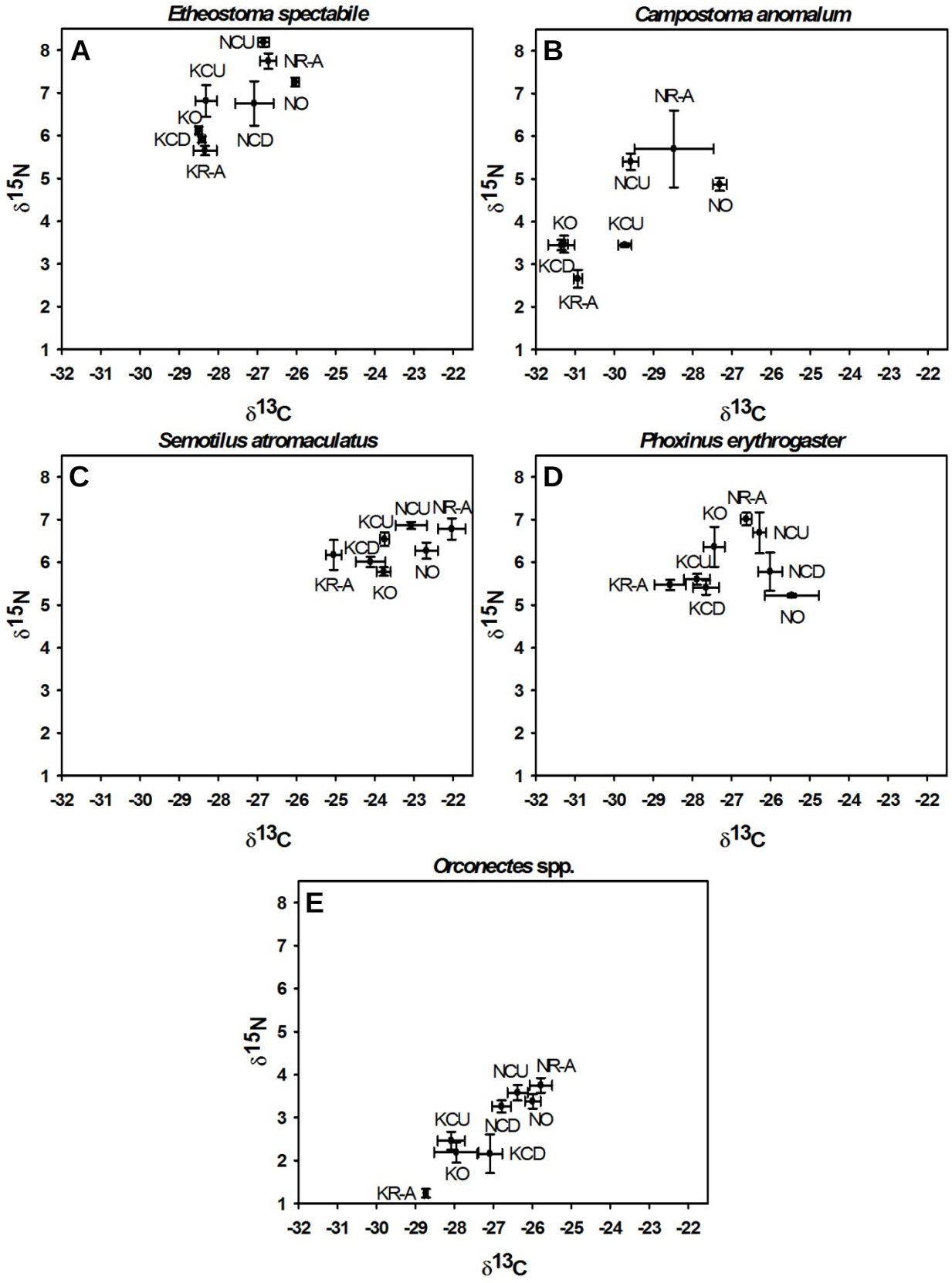
Etheostoma spectabile
A
NCU
NR-A
KCU
NO
KO
KCD
NCD
KR-A
Campostoma anomalum
B
NR-A
NCU
NO
KO
KCU
KCD
KR-A
Semotilus atromaculatus
C
NCU
NR-A
KCU
KCD
NO
KR-A
KO
Phoxinus erythrogaster
D
NR-A
KO
NCU
KCU
NCD
KR-A
KCD
NO
Orconectes spp.
E
NCU
NR-A
KCU
NCD
NO
KCD
KO
KR-A
δ15N
δ13C
1
2
3
4
5
6
7
8
-32
-31
-30
-29
-28
-27
-26
-25
-24
-23
-22

Figure 4.9 Difference in $\delta^{13}C$ values between spring 2007 and 2009 for shredders (A). Difference in $\delta^{13}C$ values between spring 2007 and 2009 for *Etheostoma spectabile* (B).

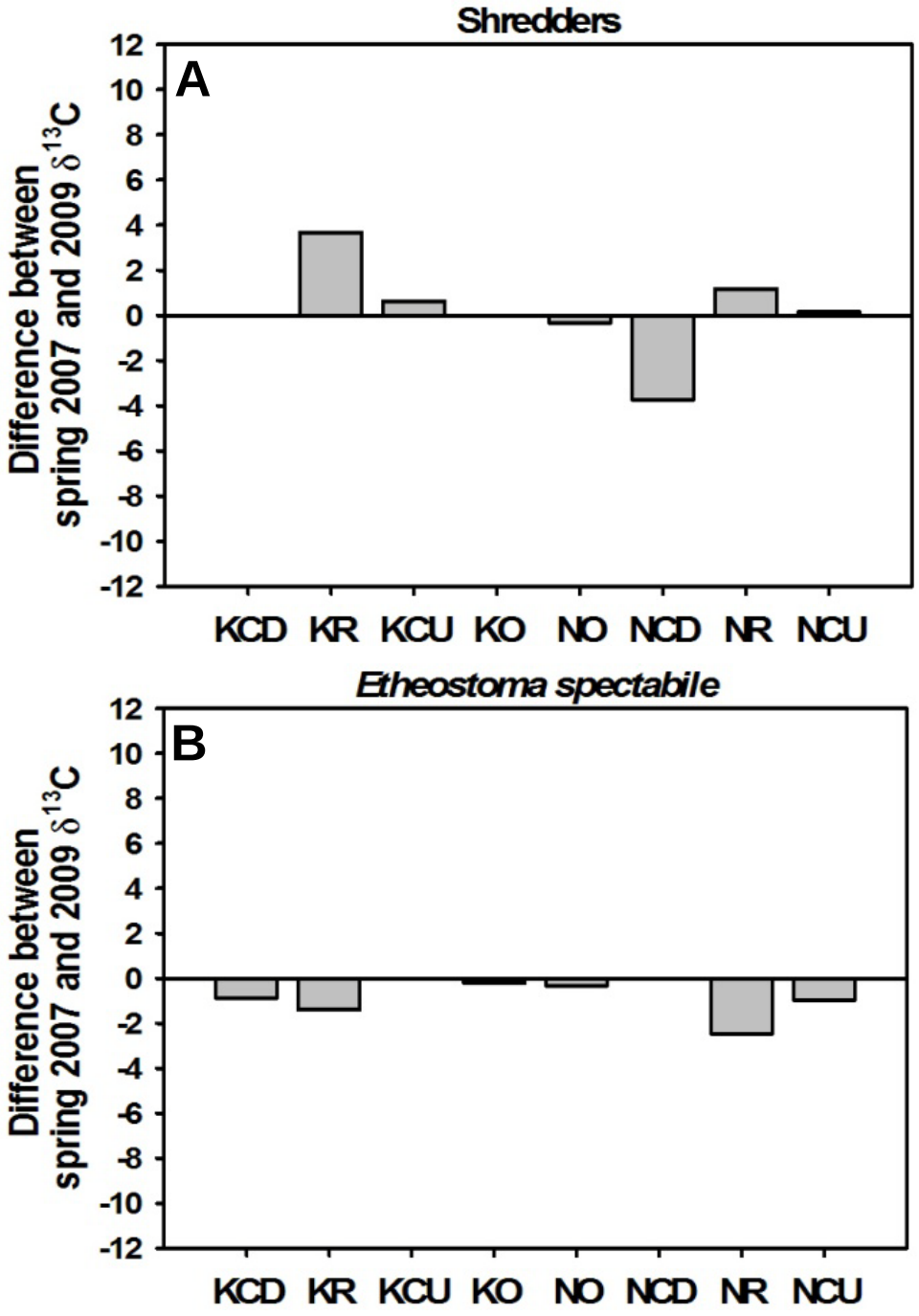

Figure 4.10 Difference in $\delta^{15}N$ values between spring 2007 and 2009 for shredders (A) and scrapers (B). Difference in $\delta^{15}N$ values between summer 2007 and 2009 for predators (C) and *Orconectes* spp. (D).

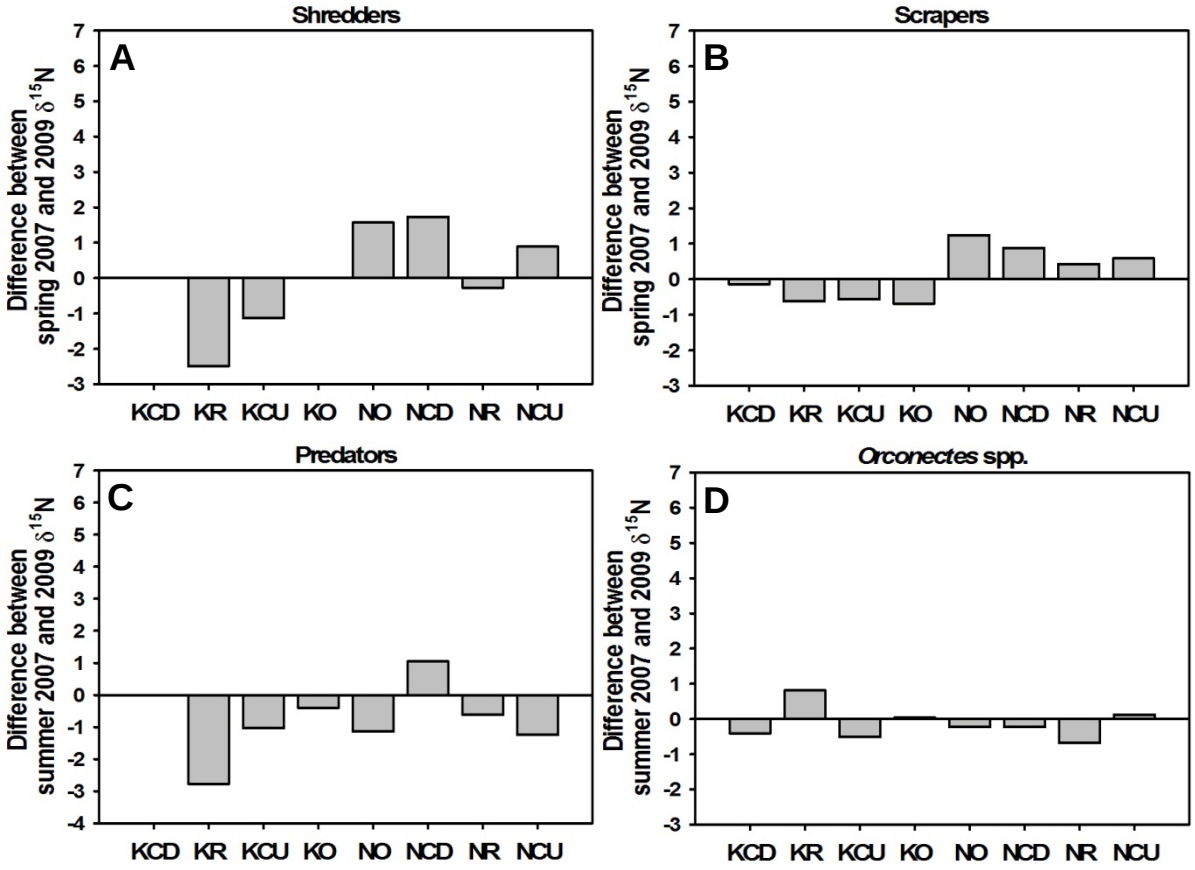

Table 4.1 ANCOVA results for 8 reaches in Kings Creek with leaf material as the dependent variable, season and watershed as categorical variables, and days since flood and % canopy as continuous variables. Significant results have a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	2528.5	1	2528.5	0.286	0.596
days since flood	1141.6	1	1141.6	0.129	0.721
% canopy	97668.4	1	97668.4	11.046	0.002*
season	271689.5	2	135844.8	15.364	< 0.001*
watershed	750.9	1	750.9	0.085	0.772
season*watershed	23024.1	2	11512.0	1.302	0.283
error	353670.4	40	8841.8		

Table 4.2 Kendall Tau correlation analysis of percent canopy for 8 reaches in Kings Creek compared to filamentous algal biomass and chlorophyll *a* response variables. Significant results have a p-value < 0.05 and are denoted by an asterisk (*).

Comparison	Kendall Tau	p-level
canopy vs. filamentous algal biomass	-0.243	0.015*
canopy vs. chlorophyll *a*	-0.143	0.153
canopy vs. filamentous:chlorophyll ratio	-0.214	0.032*

Table 4.3 ANCOVA results for 8 reaches in Kings Creek with filamentous algal biomass as the dependent variable, season and watershed as categorical variables, and days since flood and % canopy as continuous variables. Significant results have a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	7.318	1	7.3184	0.263	0.611
days since flood	116.190	1	116.1898	4.179	0.048*
% canopy	220.208	1	220.2079	7.920	0.008*
season	83.477	2	41.7384	1.501	0.235
watershed	24.895	1	24.8949	0.895	0.350
season*watershed	9.080	2	4.5402	0.163	0.850
error	1112.118	40	27.8030		

Table 4.4 Kendall Tau correlation analysis of the number of days since flood (dsf) for 8 reaches in Kings Creek compared to reach response variables with significant results having a p-value < 0.05 and denoted by an asterisk (*).

Comparison	Kendall Tau	p-level
dsf vs. wood material	0.107	0.285
dsf vs. leaf material	0.227	0.023*
dsf vs. filamentous algal biomass	0.338	0.001*
dsf vs. chlorophyll *a*	0.193	0.054
dsf vs. filamentous:chlorophyll ratio	0.352	< 0.001*

Table 4.5 ANCOVA results for 8 reaches in Kings Creek with chlorophyll *a* as the dependent variable, season and watershed as categorical variables, and days since flood and % canopy as continuous variables. Significant results have a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	2047.424	1	2047.424	61.826	< 0.001*
days since flood	528.322	1	528.322	15.954	< 0.001*
% canopy	198.309	1	198.309	5.988	0.019*
season	841.014	2	420.507	12.698	< 0.001*
watershed	277.078	1	277.078	8.367	0.006*
season*watershed	89.820	2	44.910	1.356	0.269
error	1324.632	40	33.116		

Table 4.6 ANCOVA results for 8 reaches in Kings Creek with filamentous algal biomass:chlorophyll *a* ratio as the dependent variable, season and watershed as categorical variables, and days since flood and % canopy as continuous variables. Significant results have a p-value < 0.05 and are denoted by an asterisk (*).

	SS	df	MS	F	p
Intercept	2138.32	1	2138.32	1.868	0.179
days since flood	10006.07	1	10006.07	8.739	0.005*
% canopy	8550.71	1	8550.71	7.468	0.009*
season	6027.23	2	3013.61	2.632	0.084
watershed	1329.23	1	1329.23	1.161	0.288
season*watershed	213.02	2	106.51	0.093	0.911
error	45799.02	40	1144.98		

Table 4.7 Range and average values of $\delta^{13}C$ and $\delta^{15}N$ for food sources collected from 8 reaches in Kings Creek during the spring and summer of 2007 and 2009.

	$\delta^{13}C$		$\delta^{15}N$	
Food Source	Range	Average	Range	Average
Leaves	-29.58 to -26.68	-28.41	-2.65 to 0.28	-1.17
Filamentous algae	-33.00 to -15.79	-25.84	-1.59 to 1.61	-0.12
Bryophytes	-24.95 to 12.01	-19.60	-1.57 to 0.99	-0.22
Epilithon	-27.61 to -7.26	-16.48	0.13 to 7.39	2.66
FBOM	-19.26 to -9.86	-14.79	0.12 to 4.09	1.75

Chapter 5 - Conclusion

The encroachment of woody vegetation along prairie stream corridors is a landscape change that is occurring rapidly in many areas of remaining tallgrass prairie in North America. An assessment of how this change in canopy cover will affect prairie streams is important because there are few prairie streams remaining that originate from relatively native grasslands. The research presented in this dissertation investigated methods for measuring whole-stream metabolism as a necessary precursor to studying how woody vegetation encroachment influences prairie stream ecosystems.

The second chapter explored components of estimating metabolic rates, including reach length requirement for two-station metabolism, aeration rate estimates, and temperature effect on metabolism. I found that experimental reaches of at least 20 m were necessary to estimate effects of riparian cover on metabolism, and models that estimate whole-stream metabolic rates are most accurate if they include temperature effects on metabolism. Analysis of precise O_2 measurements revealed that approximately 20 m is the minimum reach length required before significance can be obtained for metabolism measurements made using the two-station method in streams of similar biological and hydrological characteristics. The two-station method is necessary to measure metabolism in a defined reach, as I used in the following two chapters. The length of 20 m is comparable to the median length of 25 m obtained from an equation from Reichert et al. (2009) that includes aeration and velocity. Accurate aeration rates can be difficult to obtain, therefore it is beneficial to have a method for determining minimum reach length required without having to measure aeration. Measured and modeled aeration were compared from 16 separate measurements. The non-linear curve fitting model presented in this dissertation was somewhat successful at predicting aeration. The model was also used to compare measured

and modeled O_2 values to determine if the fit was improved by temperature correcting metabolic rates. Temperature corrected metabolism resulted in a better fit between measured and modeled values, suggesting that estimates of metabolic rate should be corrected for temperature. Temperature correcting metabolism would also allow for better cross-site comparisons of rates.

The third chapter describes the investigation of how metabolism was influenced by various degrees of canopy cover. Two-station metabolism was measured in closed canopy, naturally open canopy, and vegetation removal reaches. Before removal of woody riparian vegetation, rates of community respiration increased as canopy cover increased, and stream reaches became more net heterotrophic (i.e. had a greater heterotrophic state with a shift toward dominance by respiratory metabolism). However, this trend was not apparent after vegetation removal. A longer time period or greater reach length of removal may be necessary to determine the full effect of canopy removal. Canopy cover did not affect gross primary production rates across all types of canopy. However, removal of canopy cover did slightly increase gross primary production in the vegetation removal reaches when rates from before removal were compared to rates after the removal. One removal reach displayed substantial increases in gross production and the other one was not affected. The differential responses were attributed to differences in reach orientation, with watershed K02A responding more strongly and being less shaded by nearby hill topography. Chlorophyll *a* concentration was greater in open canopied reaches than close canopied reaches when chlorophyll concentrations were combined by season and year.

The fourth chapter details my study of the effects of woody vegetation encroachment on the standing stock of wood and leaves, filamentous algal biomass, and chlorophyll *a* concentration in Kings Creek as an extension of the metabolism experiment (i.e. ecosystem

structure in addition to ecosystem function). Food web interactions were compared to detect shifts in interactions between reaches. Leaf and wood material were greater in reaches with riparian canopy cover, although the removal reaches were manipulated to decrease the amount of material allowed to enter, and open canopy reaches would be expected to have low amounts of wood and leaves. Standing stock differences between the closed canopy and open canopy reaches demonstrated that the treatment effect of the removal created conditions similar to those in the naturally open canopy reaches. Measurements of leaf and wood material also showed that the closed canopy reaches had more material than the naturally open canopy reaches, and that these differences were much more pronounced in fall immediately following leaf loss of deciduous riparian trees and shrubs. Filamentous algal biomass decreased with an increase in canopy cover, as did chlorophyll *a*, however chlorophyll was affected to a lesser extent. Both filamentous algal biomass and chlorophyll were significantly impacted by the number of days since the previous flood. Chlorophyll was affected more than filamentous algal biomass by floods. A decrease in the filamentous algal biomass to chlorophyll ratio with an increase in canopy cover indicated a shift in algal community structure in open reaches. Fewer filaments present in closed canopy reaches could influence the type of consumers present due to a change in available food sources.

The fourth chapter also explored stable isotope analysis to indicate consumer food web interactions. The $\delta^{13}C$ and $\delta^{15}N$ values of food sources collected from all reaches had a high degree of overlap, particularly for filamentous algal biomass and deciduous tree leaves (the ecosystem compartments that were most influenced by vegetation removal). The overlap in signatures made it difficult to conclude if consumers had shifted their diet towards filamentous algae (associated with open canopy reaches) or if they shifted towards leaves (associated with

closed canopy). However, analyses of food sources did rule out a shift to epilithon or fine benthic organic matter, which had a significantly different isotopic composition from leaves and filamentous algae. A comparison of $\delta^{13}C$ and $\delta^{15}N$ from four fish species and crayfish from one sampling period in August of 2009 revealed that each species grouped together with some overlap among *Etheostoma spectabile*, *Phoxinus erythrogaster*, and *Campostoma anomalum*. *E. spectabile* is an invertivore, and its overlap with other species would indicate that those species were also consuming invertebrates. Most of the time *P. erythrogaster* are herbivores, but young fish may eat some invertebrates, suggesting that during this time of year the community structure of this species is comprised mostly of younger fish. *C. anomalum* tends to be omnivorous, and the isotope results suggested that the main food source for *C. anomalum* could be different between the two subwatersheds.

Isotope analysis from the summer of 2009 also revealed that crayfish were eating filamentous algae or leaves and most likely not invertebrates. These data suggest that crayfish were able to switch food sources with changes that were associated with removal of woody vegetation. Crayfish can also eat detritus and be predators, but these were apparently not important food sources in this study.

Overall, the data presented in this dissertation give insight into how woody vegetation encroachment affects a prairie stream ecosystem both structurally and functionally. As woody vegetation encroachment continues to close in the canopy in these headwater streams, the effects may become even more pronounced. The removal reaches were relatively short, so greater lengths of riparian cover could have even more prominent effects. Future research should investigate effects over longer reaches or entire subwatersheds. My results indicate that restoration by removing canopy cover does restore natural conditions to some degree. It would

be best to protect and preserve prairie streams before restoration efforts would be needed, but restoration may be a viable option if managers are interested in conserving prairie streams in their native state. Research on spatial scale of canopy removal and longer-term responses would provide valuable information for such restoration techniques.

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Appendix A - Supplemental material to Chapter 4

Figure A.1 Difference in filterer $\delta^{13}C$ values between 2007 and 2009 for spring (A) and summer (B). Difference in filterer $\delta^{15}N$ values between 2007 and 2009 for spring (C) and summer (D).

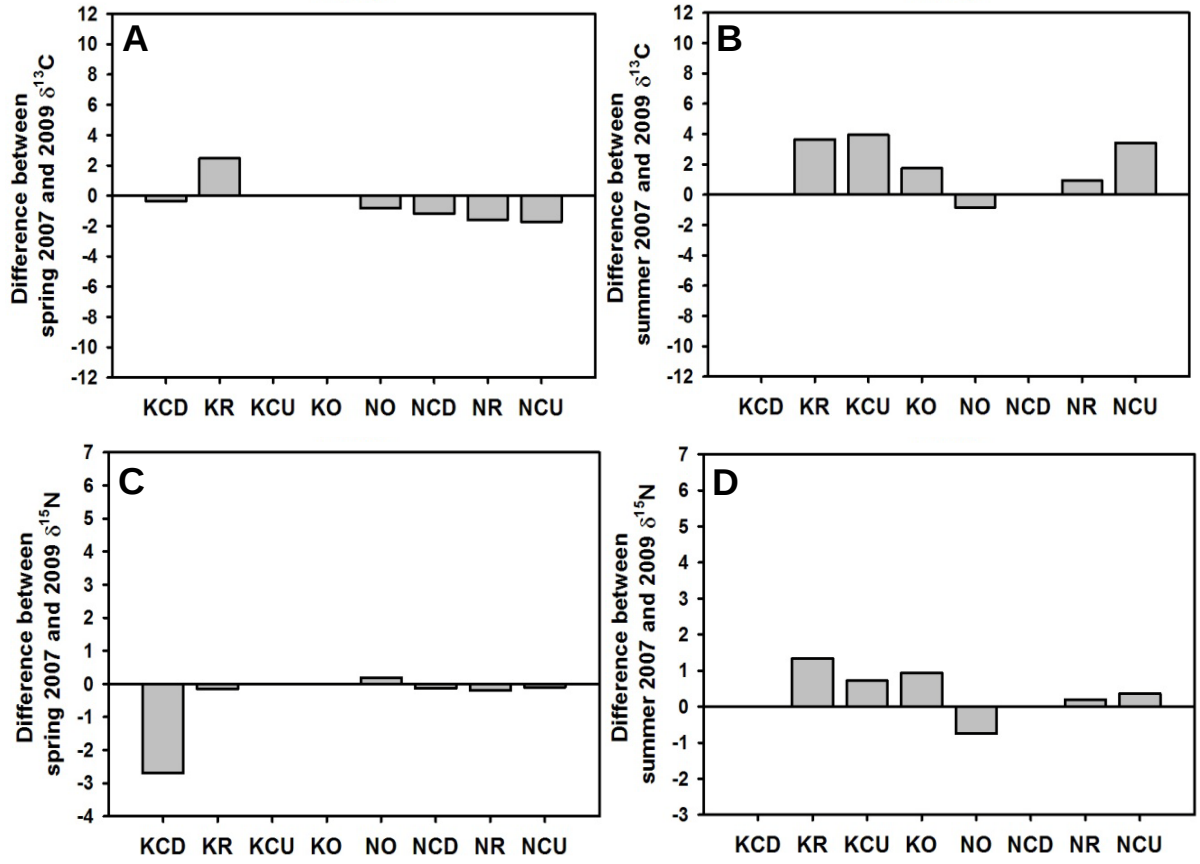

Figure A.2 Difference in predator $\delta^{13}C$ values between 2007 and 2009 for spring (A) and summer (B). Difference in predator $\delta^{15}N$ values between 2007 and 2009 for spring (C).

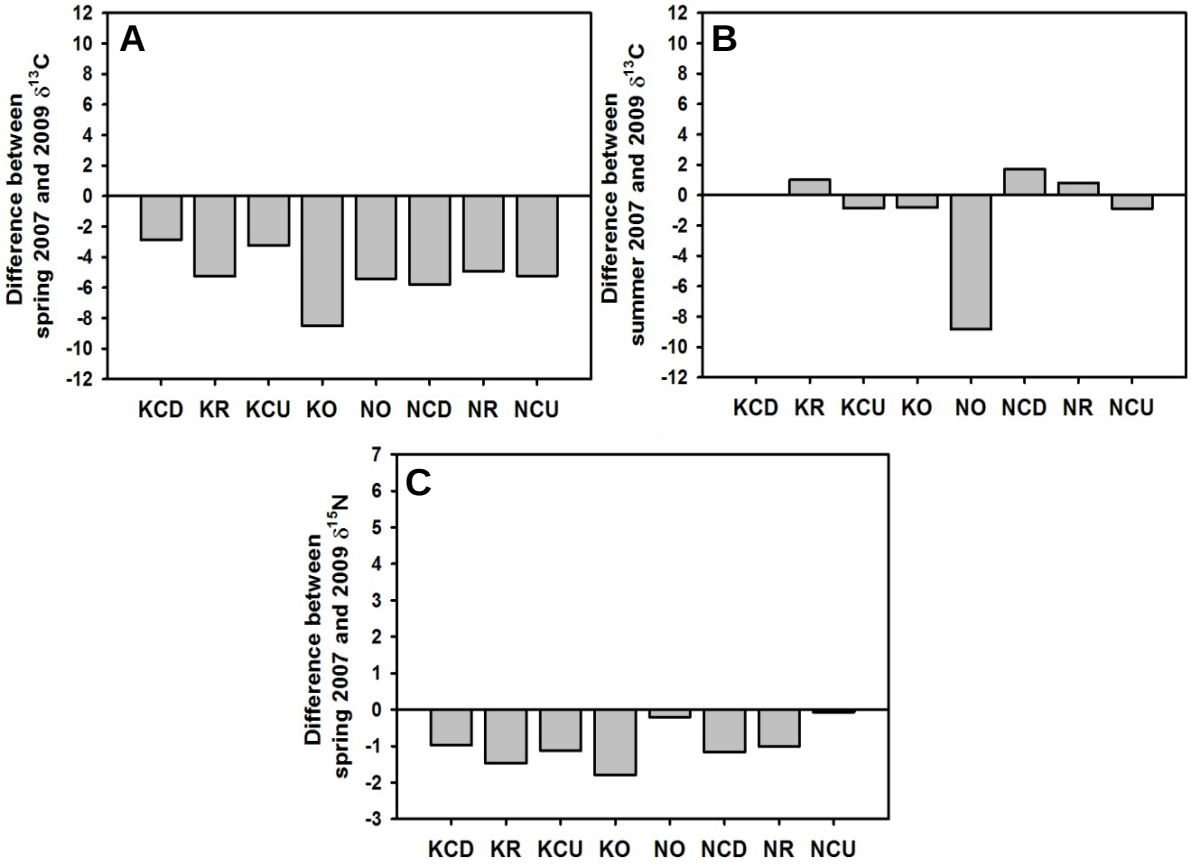

Figure A.3 Difference in scraper $\delta^{13}C$ values between 2007 and 2009 for spring (A) and summer (B). Difference in scraper $\delta^{15}N$ values between 2007 and 2009 for summer (C).

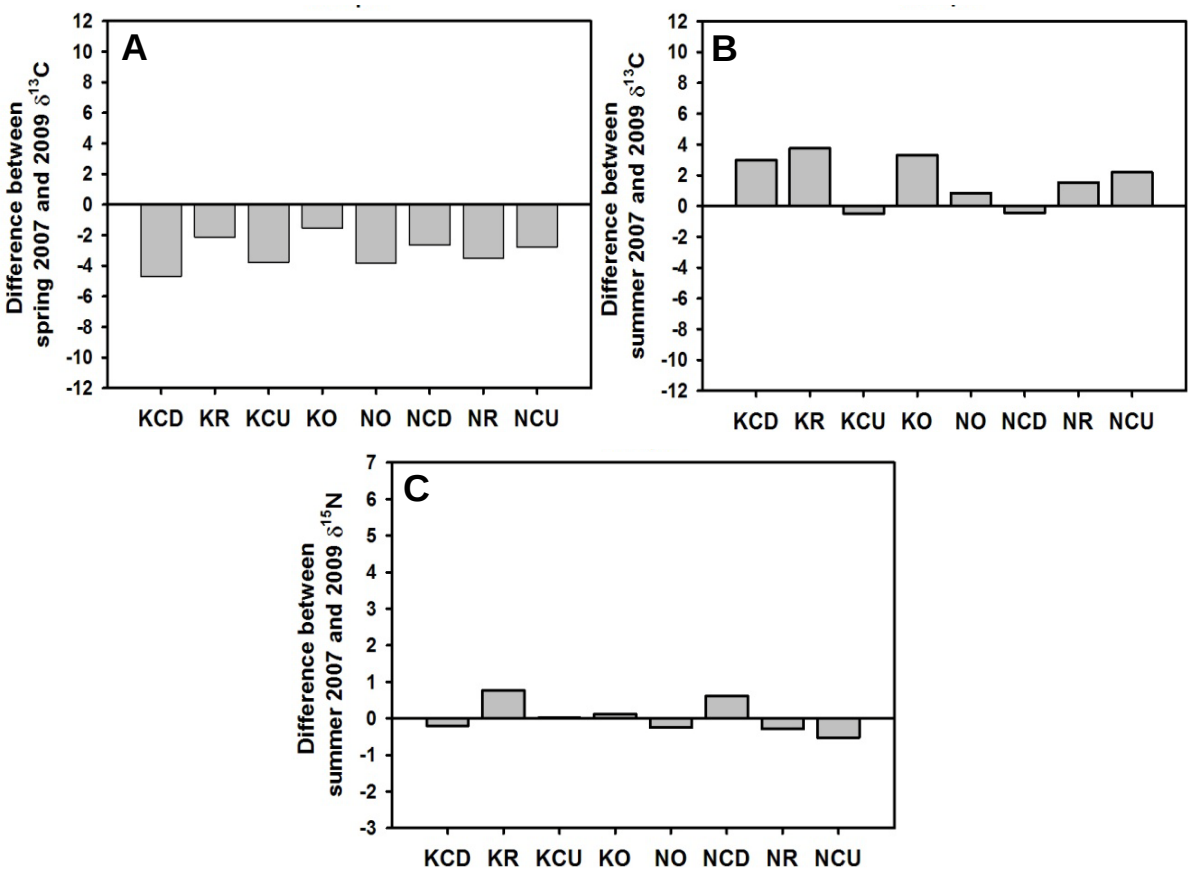

Figure A.4 Difference in *Orconectes* spp. $\delta^{13}C$ values between 2007 and 2009 for spring (A) and summer (B). Difference in *Orconectes* spp. $\delta^{15}N$ values between 2007 and 2009 for spring (C).

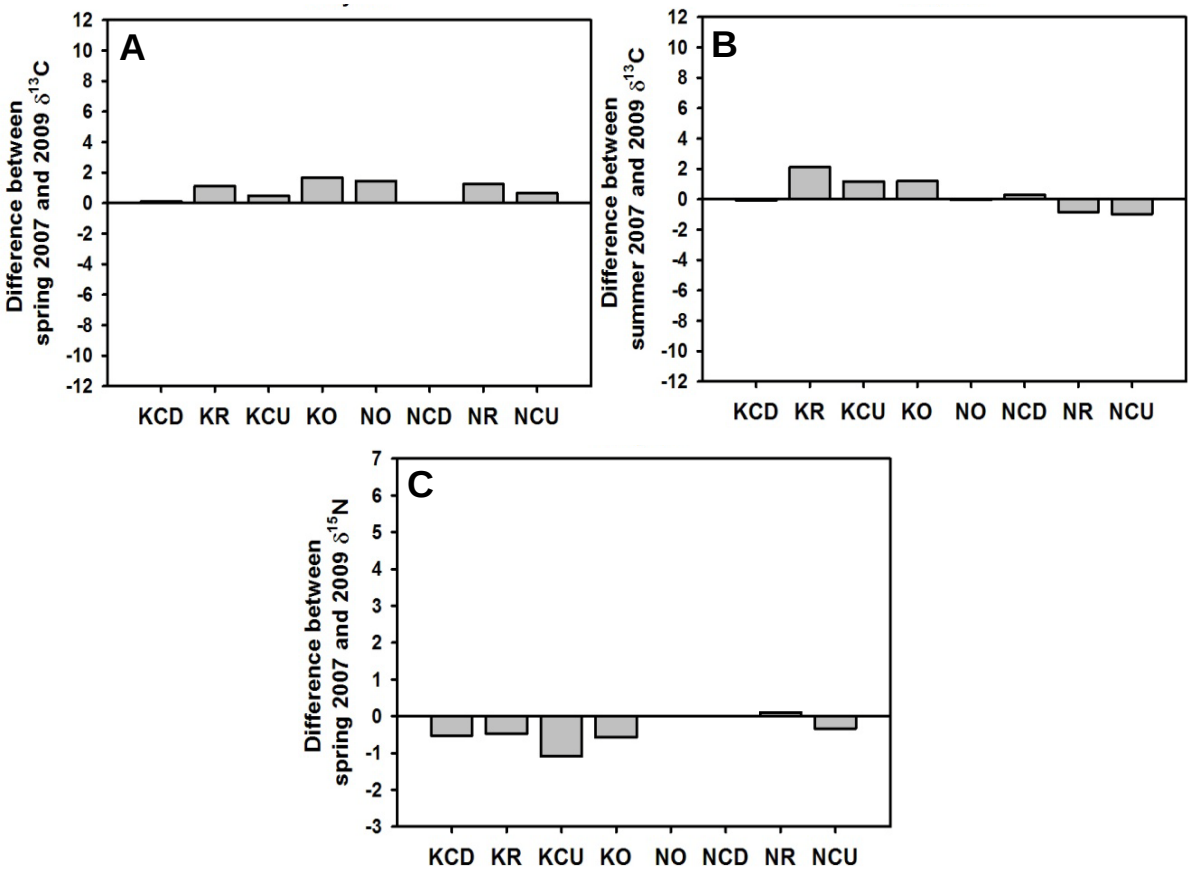

Figure A.5 Difference in *Etheostoma spectabile* $\delta^{13}C$ values between 2007 and 2009 for summer (A). Difference in *Etheostoma spectabile* $\delta^{15}N$ values between 2007 and 2009 for spring (B) and summer (C).