

The expansion of woody riparian vegetation, and subsequent stream restoration, influences the metabolism of prairie streams

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SUMMARY

1. Tallgrass prairies and their streams are highly endangered ecosystems, and many remaining streams are threatened by the encroachment of woody riparian vegetation. An increase in riparian vegetation converts the naturally open-canopy prairie streams to closed-canopy systems. The effects of a change in canopy cover on stream metabolism are unknown.

2. Our goal was to determine the effects of canopy cover on prairie stream metabolism during a 4-year period in Kings Creek, KS, U.S.A. Metabolic rates from forested reaches were compared to rates in naturally open-canopy reaches and restoration reaches, the latter having closed canopies in 2006 and 2007 and open canopies in 2008 and 2009. Whole-stream metabolism was estimated using the two-station diurnal method. Chlorophyll *a* concentrations and mass of filamentous algae were measured after riparian removal to assess potential differences in algal biomass between reaches with open or closed canopies.

3. Metabolic rates were spatially and temporally variable even though the sites were on very similar streams or adjacent to each other within streams. Before riparian vegetation removal, whole-stream community respiration (CR) and net ecosystem production were greater with greater canopy cover. In the vegetation removal reaches, gross primary production was slightly greater after removal.

4. Chlorophyll *a* concentrations were marginally significantly greater in open (naturally open and removal reaches) than in closed canopy and differed significantly between seasons. Filamentous algal biomass was greater in open than in closed-canopy reaches.

5. Overall, the restoration allowed recovery of some features of open-canopy prairie streams. Woody expansion apparently increases CR and moves prairie stream metabolism towards a more net heterotrophic state. An increase in canopy cover decreases benthic chlorophyll, decreases dominance of filamentous algae and potentially alters resources available to the stream food web. The results of this study provide insights for land managers and conservationists interested in preserving prairie streams in their native open-canopy state.

Keywords: macroalgae, microalgae, primary production, restoration, streams

Introduction

North American tallgrass prairie has been significantly altered by human activity, resulting in the loss of more than 95% of prairie lands (Samson & Knopf, 1994). In many areas where prairie has not been ploughed for cropland, contemporary control of fires has encouraged the growth of woody vegetation. Historically, owing to

the frequency of wildfires, ungulate grazing and hydrological patterns, low-order prairie streams were commonly characterised by open canopies associated with riparian grass cover (Dodds *et al.*, 2004). Now, the riparian vegetation in many remaining tallgrass streams is becoming increasingly similar to that in deciduous forests. Riparian cover of headwater streams could influence downstream biogeochemical properties by altering

sunlight availability and system inputs (Dodds & Oakes, 2008). An increase in canopy cover decreases the amount of sunlight, which could in turn lower water temperatures. Nutrient concentrations could be impacted by a change in canopy cover because leaf and woody material input could change carbon and nitrogen availability. Coarse allochthonous inputs from woody canopies are greater than those from grassy riparian areas and dominated by carbon-rich leaves (Stagliano & Whiles, 2002; Dodds *et al.*, 2004). Thus, basic ecosystem structure and function could be changed fundamentally by woody expansion in the riparian zones of prairie streams.

Stream metabolism indicates ecosystem energy flux and is related to downstream water quality because it is intimately tied to in-stream nutrient processing (Fellows *et al.*, 2006). Net ecosystem production (NEP) is based on the balance of gross primary production (GPP) and community respiration (CR), and these metabolic rates are a measure of whole-ecosystem production. Whole-stream metabolism can indicate the trophic status of a stream. Different portions of the food web depend on heterotrophic microbial components or autotrophic components of the stream. Thus, it is important to understand both CR as a measure of heterotrophic state and GPP as a measure of autotrophic state (Dodds, 2006).

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 *c.* 70% from 1939 to 2002 on Konza. Much of this expansion has occurred along Kings Creek, a prairie stream draining tallgrass prairie in Konza. Understanding metabolic processes and restoration of prairie streams via riparian vegetation removal could aid in protecting this threatened system. We had three main questions: (i) How does woody vegetation encroachment, and thus a change in canopy cover, affect prairie stream metabolism? (ii) What are the seasonal patterns of metabolism in prairie streams, and do these patterns vary with canopy type? (iii) Does restoration of moderately long reaches to open-canopy restore prairie stream ecosystem function to be more similar to natural open-canopy reaches?

To draw conclusions on the effects of canopy cover, a space-for-time substitution was used. An experimental manipulation was used to draw conclusions on effects of restoration. Here, we define restoration as returning the fundamental processes to rates similar to the native state (Bradshaw, 1997). We removed riparian woody vegetation from stream reaches and determined whether this led to whole-stream metabolic characteristics typical of native,

naturally open-canopy reaches. We hypothesised that reaches with naturally open or removed canopy would have greater GPP than reaches with a closed canopy. We predicted that GPP in open reaches would be greatest during summer when longer periods of sunlight would promote more primary production and 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 conducted during 2006–09 in eight stream reaches in two branches of an intermittent prairie stream, Kings Creek, whose catchment is encompassed within Konza. Konza is a 3487-ha tallgrass prairie that is in the northern part of the Flint Hills region near Manhattan, Kansas, in Riley County. A detailed site description has been published previously (Gray & Dodds, 1998; Gray *et al.*, 1998). Study reaches were located in two separate subcatchments (Fig. 1), N04D and K02A/AL (hereafter referred to as K02A). N04D is continuously grazed by *Bos bison* (American 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 (adjacent to K02A and the location of three of the four measurement reaches in the K02A/AL subcatchment) was burned in 2009 and is not burned on a regular burn schedule.

Study areas consisted of four reaches in each subcatchment, stratified by differences in canopy cover that either occurred naturally or were altered experimentally (Fig. 1). The site designations indicate condition of each reach. The first letter indicates K02A (K) or N04D (N) as the subcatchment. The second letter indicates naturally open canopy (O), closed riparian canopy (C) or closed canopy removed experimentally (R). The third letter indicates closed-canopy reaches 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, but other reaches were contiguous. 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 between reaches. Both NO and KO were dominated by *Andropogon gerardii* (big bluestem) and *Sorghastrum nutans* (Indian grass). These reaches also contained *Ambrosia psilotachya* (western ragweed), *Cornus drummondii* (rough-leaved dogwood) and

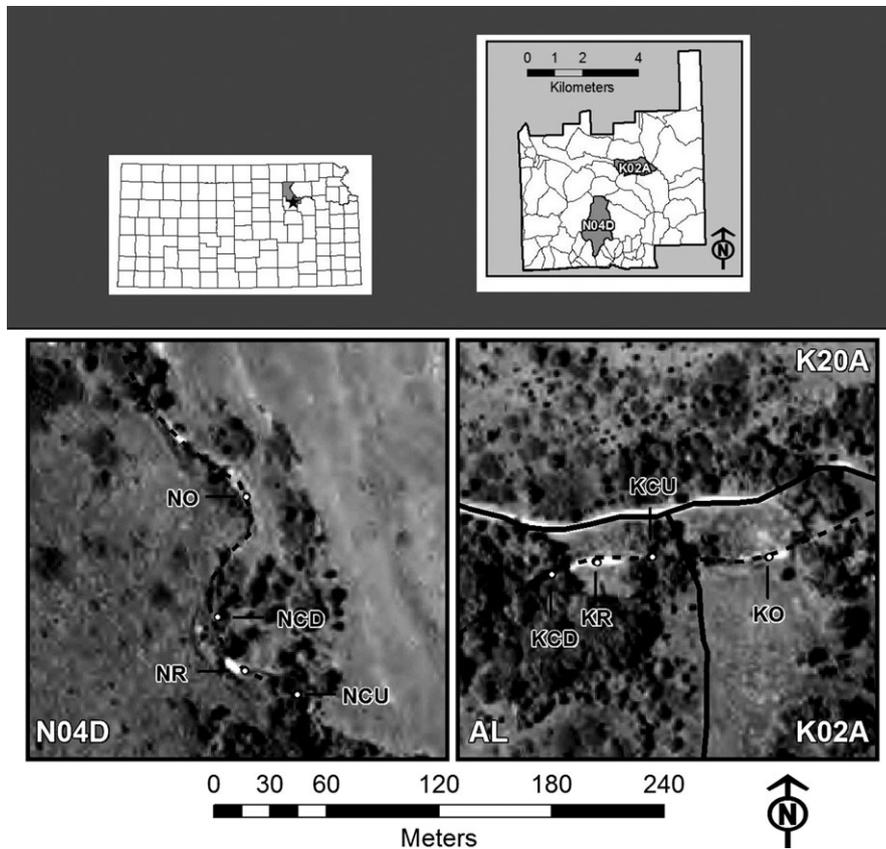


Fig. 1 Map of Kansas (top left) showing the location of Konza Prairie Biological Station within Riley county (marked with a star) and map of Konza Prairie (top right) showing study subcatchments. Aerial photographs of study sites on Konza Prairie Biological Station (bottom images). Image on the left is of grazed subcatchment N04D, and image on the right is of study reaches located in ungrazed subcatchments K02A/AL. The stream channel is marked with a dashed line, and individual reaches are represented by circles placed at the midpoint of the reach along with the reach code. Stream flow is from south to north in N04D and from east to west in K02A/AL. Images are courtesy of Adam Skibbe.

numerous perennial forbs. NR-B, NCU and NCD were dominated by *Ulmus americana* (American elm) and *Gleditsia triacanthos* (honey locust). KR-B, KCU and KCD were dominated by *Quercus macrocarpa* (bur oak) and *Quercus muehlenbergii* (chinquapin oak). All closed-canopy reaches in both subcatchments had an understory that consisted of various grass and forb species. Following woody removal, NR-A was comprised mostly of *Bromus japonicus* (Japanese brome), western ragweed and dogwood patches. KR-A consisted of more woodland understory species than NR-A. The most prominent species were *Parthenocissus quinquefolia* (Virginia creeper), *Andrachne phyllanthoides* (buckrush) and *Sanicula canadensis* (black snakeroot). Although riparian vegetation differed between the reaches, all eight reaches were similar in stream characteristics such as depth, width, velocity and discharge (Table 1).

Experimental manipulation

Woody vegetation was removed from a 30-m buffer around the stream in the two removal reaches in December 2007

(Fig. 2). The removal took place during the winter on frozen ground to minimise impact to the riparian area. Larger trees were removed with a chainsaw, and smaller vegetation was removed by 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 regrowth. Wood that was removed was pulled away from the stream and out of the removal area. The open canopy associated with the removal of vegetation was maintained throughout the remainder of the study by mowing and manual clipping every winter. We would not expect longer naturally open reaches to receive large subsidies of leaves from upstream. Therefore, prior to leaves falling from trees in the autumn, a wire mesh fence with 1-cm holes was placed across the entire stream on the upstream side of the removal reach to collect leaves and keep them out of the removal area. 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

Table 1 Average values of site characteristics for stream reaches during the study period (2006–09). Reaeration values (k) were corrected to 20 °C. Before and after vegetation removal percentage canopy values are displayed for the removal reaches (NR and KR). For meaning of acronyms, see Methods

Reach	Length (m)	Depth (m)	Width (m)	Reaeration k_{20} (min^{-1})	Velocity (m s^{-1})	Discharge ($\text{m}^3 \text{s}^{-1}$)	Canopy (%)
NCU	22.5	0.08	1.33	0.024	0.046	0.004	66
NR	33	0.11	1.45	0.044	0.031	0.005	61/7
NCD	36	0.10	1.11	0.050	0.068	0.008	65
NO	63.5	0.08	1.72	0*	0.042	0.005	7
KO	29	0.13	2.45	0.019	0.020	0.006	20
KCU	28.5	0.04	3.35	0.031	0.026	0.006	85
KR	35.5	0.12	3.06	0.027	0.045	0.015	83/4
KCD	27	0.20	4.02	0.030	0.026	0.019	80

* k was set to zero if not significantly different from zero ($P < 0.05$).

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 reaeration

Metabolism was estimated when differences in canopy cover were expected to have the greatest influence. We estimated metabolism at baseflow to indicate average conditions and to allow reaeration rates to apply in cases when they were not made at precisely the same time. Metabolism was estimated 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 autumn/winter after the leaves fell for each year. Metabolism rates were estimated 4–5 times a year for 4 years (2006–09) using the 2-station upstream–downstream method (Marzolf, Mulholland & Steinman, 1994; Young & Huryn, 1998) to isolate metabolism of specific stream reaches. All reaches within each subcatchment were measured on the same day. March, April and May estimates

were classified as spring samples. June, July, August and September were summer estimates, and October, November and December were autumn estimates.

Yellow Springs Instruments, Inc. (Yellow Springs, OH, U.S.A.) logging data sondes (model 6600 with optical or 600 with membrane probes) were used to record O_2 and temperature values every 10 min. Before deployment, all sondes were calibrated together in the field. Sondes were placed in the stream for 30 min to be certain that all sondes were at 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 water-saturated air and placed back in the stream and allowed to log for 30 min. 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 min. 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.

Every set of diel data from each sonde was first checked to make sure that the sonde had worked properly. Some diel data had to be discarded owing to equipment malfunction or owing 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. Once raw diel data passed the first inspection, metabolic rate was modelled.

Reaeration was measured to allow accurate modelling of whole-stream metabolism. Reaeration measurements were taken at baseflow during 2006, 2007 and 2009. Attempts to obtain reaeration rates in 2008 failed owing to gas chromatography problems. Reaeration was measured in the same reaches where diurnal O_2 measurements were taken using a tracer gas (propane) and an inert tracer dye (rhodamine) or ion (bromide) in all eight reaches (i.e. reaeration rates were measured for every reach). Details of



Fig. 2 Grazed removal reach in N04D subcatchment (NR) (a) before vegetation removal in August 2007 and (b) after removal in August 2008.

reaeration determination methods can be found in appendices associated with the study of Mulholland *et al.* (2008).

Replicates of gas samples were taken at each sonde placement point (i.e. top and bottom of each reach) so that a reaeration rate could be determined for each of the eight reaches where metabolism was estimated. To collect gas samples, 40 mL of stream water was collected in a 60-mL syringe that had a 3-way stopcock attached. Then, 20 mL of helium gas (gas chromatography carrier gas) was injected and the syringe was shaken for 3 min. The headspace gas in the syringe was injected into an evacuated vial (vacutainer, 15 mL). The water from the syringe was analysed for tracer ion concentration or dye fluorescence to account for the dilution on each sample.

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

Additional data required for the modelling metabolism included individual reach characteristics (length, depth, width, average velocity and discharge), barometric pressure, reaeration rate and light. Width measurements were taken every few metres along the length of the reach, five depth measurements were taken across each width transect, and this was repeated during each reaeration measurement. Light values were measured using an Li-Cor LI-1000 datalogger equipped with a PAR sensor. Light measurements were logged every 10 min (corresponding with the O₂ 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. Barometric pressure was taken from a weather station (Manhattan, KS, U.S.A.), 10 km from the site.

Estimation of metabolic rates

We estimated metabolism using a model that altered rates of GPP and CR to minimise the variance between measured and modelled O₂ values. Standard calculation techniques (e.g. night-time regression) do not account for wide temperature swings common in these streams, leading to error (Riley, 2011). Temperature and O₂ data were offset by the reach-specific calculated travel time. The basic modelling approach was to calculate change in O₂ every 10 min as influenced by rates of GPP₂₀, CR₂₀ and reaeration₂₀, each rate corrected for temperature at each

time step (see Dodds *et al.*, 2008). Diel CR was corrected for temperature according to Parkhill & Gulliver (1999). GPP was set as a function of light with a hyperbolic tangent function (Jassby & Platt, 1976) and corrected for temperature as in the study of Parkhill & Gulliver (1999). Microsoft Excel 2003 solver function was used to minimise the sum of squares of error between modelled and measured values to find the best fit of our modelled O₂ to observed O₂ by changing the basic rates of GPP and CR (see Riley (2011) for variables and equations used in model). CR is presented as a negative value.

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

The number of days since a 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 & Dodds, 2005). A U.S. Geological Survey gauging station (# 06879650) on Kings Creek is located directly downstream, only a few kilometres from both N04D and K02A. Discharge data from 1980 to 2009 indicated that a discharge rate of 9.8 m³ 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 2006–09. Each flood was noted, and then, the number of days that passed until the next metabolism measurement occurred was counted.

Percentage canopy was determined using a GRS densiometer. Readings were based on the presence or absence of canopy cover visible in the densiometer and were taken every two metres in each reach during the summer months of 2007–09. The percentage of presence-to-absence readings was used to determine the percentage canopy. There were a total of nine readings for every reach that were averaged for reach-specific percentage canopy.

There was an apparent increase in filamentous algae following the woody removal, and this prompted the sampling of chlorophyll and filamentous algal biomass after the vegetation removal occurred (during 2008 and 2009). 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 °C water bath for 5 min and then placed in the dark for 12 h (Sartory & 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, U.S.A.). Chlorophyll *a* concentration was measured using either a fluorometer (Turner model 112) or a Hitachi UV/VIS U-2900 spectrophotometer (Hitachi High Technologies America, Inc., Schaumburg, IL, USA). 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 & 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 collections of filamentous algae were taken from five replicate 0.25-m² quadrats with contents from each quadrat kept separate. All filaments removed from each quadrat were dried 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, U.S.A.). Non-parametric Kendall's tau correlation analysis was run using all 111 metabolism estimates as an exploratory method. A series of analysis of covariance (ANCOVA) tests were conducted to simultaneously assess categorical (e.g. year and subcatchment) and continuous (e.g. days since flood, temperature and percentage canopy cover) variables and test for interactive effects. A two-way analysis of variance (ANOVA) of CR and GPP₂₀ in the removal reaches was used to determine differences in rates before and after vegetation removal in the absence of variance introduced by including other reaches. An ANOVA was used to analyse the chlorophyll *a* results with chlorophyll as the dependent variable and season and percentage canopy cover as categorical variables. Filamentous algal biomass results were analysed using a one-way ANOVA to determine whether filament biomass significantly differed between open- and closed-canopy reaches. Note that reaches were assumed to be independent of each other even though there may be spatial autocorrelation.

Results

Temperature and flood

Temperature mainly influenced GPP, not CR. 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 diel data sets. Based on significant temperature effects, GPP₂₀ was calculated as in the study of Parkhill & Gulliver (1999) to remove the temperature effects on GPP and adequately analyse any seasonal canopy effects independent of temperature. CR did not have to be corrected to 20 °C to effectively analyse canopy effects. Temperature was similar across all eight reaches during each individual round of metabolism measurements (e.g. average temperature of adjacent reaches varied <1.1 °C in any one round of sampling for 85% of the sampling dates), signifying that temperature effects related to reach-scale canopy cover were not an important driver of whole-stream metabolism in this study. The reaches were probably too short to heat or cool differentially.

The number of days since flood was not significantly correlated with CR, GPP corrected to 20 °C or NEP (ANCOVA $P > 0.05$). Thus, days since flood data are not considered further.

Community respiration

Community respiration in the removal reaches was significantly different before and after vegetation removal when season was taken into account (Table 2), indicating that canopy affected metabolism. When all eight reaches were compared, an ANCOVA of before and after removal CR did not result in any significant correlations between subcatchments ($P = >0.05$); therefore, rates from N04D and K02A were combined. CR was separated into before and after vegetation removal and averaged within season to estimate an average rate for spring, summer and autumn (Fig. 3). During the spring and autumn after the removal, CR was lower than before the removal in the removal reaches (Fig. 3). Lower CR values occurred in spite of a trend towards increasing CR in closed-canopy

Table 2 Two-way ANOVA results from two removal reaches (NR and KR) with CR as the dependent variable and season and BR/AR (before removal/after removal) as categorical variables

	SS	d.f.	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		

Significant results had a P -value <0.05 and are denoted by an asterisk (*). SS, sum of squared of error; d.f., degrees freedom; MS, mean sum square error; F, f statistic; P , significance.

reaches after as compared to before removal. Generally, average summer CR increased after vegetation removal, but did not appear to be affected by canopy cover.

Percentage canopy cover clearly affected CR before removal, as greater percentage canopy cover led to greater rates of CR (ANCOVA; $P = 0.001$) when comparing across all eight reaches (Fig. 4; Table 3). However, during the period after vegetation removal, canopy did not significantly affect CR.

Gross primary production

GPP₂₀ was generally greatest during summer months in open reaches when periods of sunlight were maximal. The maximum GPP₂₀ during the course of the study was greatest in the summer for seven of the eight reaches (Fig. 5). However, GPP₂₀ was extremely variable, and rates were high during other seasons as well. For example, NCD had a high GPP₂₀ in the autumn of 2006, and KCD had high values in the autumn of 2008 and spring of 2009. NR, the only reach to have the maximum GPP₂₀ during autumn (2006), had the minimum rate 17 days earlier.

Contrary to expectation, removal reaches did not have the greatest GPP₂₀ after the removal. The maximum GPP₂₀ 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 autumn in October 2006. The minimum rate for NR was also in October 2006 and was about 5 times lower than the maximum. The maximum GPP₂₀ for KR was 69 times greater than the minimum and was estimated after the removal during the summer in July 2009. The minimum rate for KR was estimated before the removal during late

summer in September of 2006. KR usually had greater GPP₂₀ after the removal than before and also had the greatest difference between minimum and maximum rates across all eight reaches.

There was an increase in GPP₂₀ after the removal at KR. ANCOVA of data from just NR and KR was conducted to avoid the variance associated with the non-treatment reaches. The ANCOVA revealed that GPP₂₀ was greater with less canopy cover ($P = 0.050$; Table 4). NR did not change significantly after the removal, but GPP₂₀ for KR increased 5.6-fold after the removal (Fig. 6). The significance of the results across subcatchments is driven by the difference in GPP₂₀ before and after the removal at KR.

Factors influencing net metabolic rate and primary producer biomass

Net ecosystem production response to canopy cover was similar to the CR response. Reaches with a greater percentage of canopy cover had a greater NEP (ANCOVA; $P = 0.001$) across all eight reaches before the removal (Table 5). After removal, canopy was not significantly related to NEP.

After the removal, we observed more filaments at KR than NR. In general, open-canopy reaches had more filaments than closed-canopy reaches (Table 6). 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 influenced positively by canopy removal. A factorial ANOVA showed that chlorophyll *a* did not differ significantly across all reaches ($P = 0.274$). Therefore, reaches were combined by subcatchment and

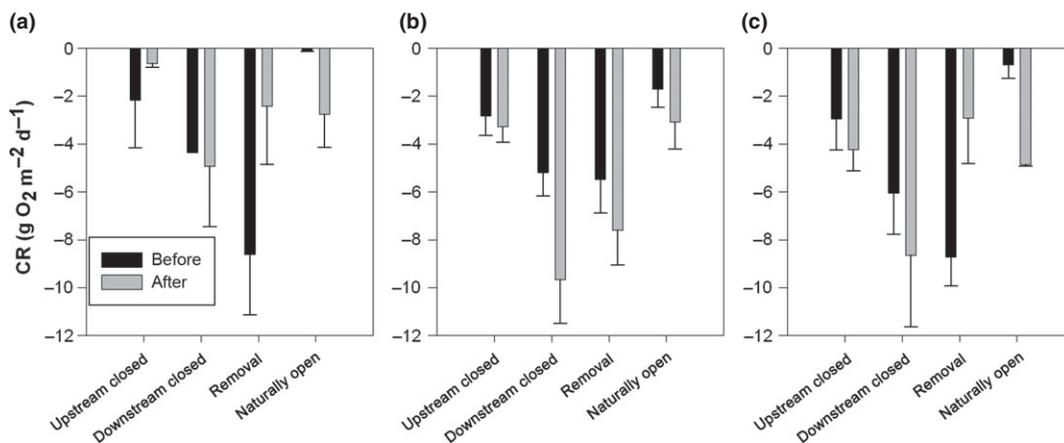


Fig. 3 CR estimated from subcatchments N04D (grazed) and K02A (ungrazed) in Kings Creek. Metabolism rates were separated into before removal rates (2006–07) and after removal rates (2008–09) and averaged for (a) spring, (b) summer and (c) autumn. Rates were combined by subcatchment (ANCOVA, $P > 0.05$ for subcatchment). Error bars represent standard error.

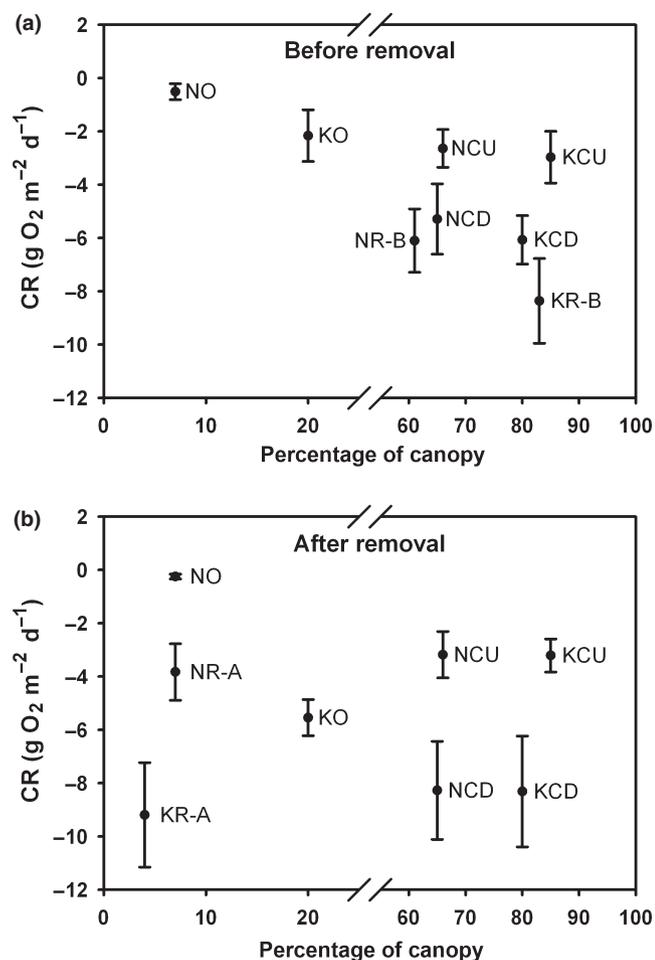


Fig. 4 Average community respiration (CR, a) before the removal (naturally open canopy: NO and KO; closed canopy: NR-B, KR-B, NCU, KCU, NCD and KCD) and (b) after the removal (open canopy: NO, KO, NR-A and KR-A; closed canopy: NCU, KCU, NCD and KCD), with percentage canopy cover for all eight reaches in Kings Creek. Before riparian vegetation removal, the greater the canopy cover, the greater the CR (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.

grouped into open or closed canopy (Fig. 7). A factorial ANOVA indicated marginally significant greater chlorophyll in open- than in closed-canopy reaches ($P = 0.057$). This test also showed that chlorophyll *a* did differ significantly between seasons ($P = 0.031$). During the spring, mean chlorophyll *a* concentration was $2.7 \mu\text{g cm}^{-2}$ greater in open- than in closed-canopy reaches. Mean chlorophyll concentrations were lower in summer, and open-canopy reaches had mean chlorophyll *a* concentration of $2.3 \mu\text{g cm}^{-2}$ more than closed canopy. Open-canopy reaches had the highest chlorophyll values in autumn, and the average chlorophyll concentration was 1.5 times greater than closed-canopy reaches.

Table 3 ANCOVA results from eight reaches in Kings Creek for 2006 and 2007 metabolism (before riparian vegetation removal) with CR as the dependent variable, season and subcatchment as categorical variables and days since flood, temperature and percentage canopy as continuous variables

	SS	d.f.	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
Percentage canopy	125.302	1	125.302	14.196	0.001*
Season	41.285	2	20.642	2.339	0.110
Subcatchment	1.572	1	1.572	0.178	0.675
Season*subcatchment	8.314	2	4.157	0.471	0.628
Error	344.225	39	8.826		

Significant results had a P -value < 0.05 and are denoted by an asterisk (*). SS, sum of squared of error; d.f., degrees freedom; MS, mean sum square error; F, f statistic; P, significance.

Discussion

Temperature and metabolism

We found neither CR nor NEP correlated with temperature across seasons and dates, indicating other factors control these rates more strongly than stream temperature. It is possible that CR is more related to hyporheic temperature, which we did not measure but would expect to be quite similar across all reaches. GPP, being driven by organisms nearer the water column, would thus be more closely related to temperature than CR. We acknowledge it can be difficult to separate the effects of temperature from light on primary production rates (Wetzel, 2001). Changes in canopy cover could cause a temperature response at the subcatchment 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. Canopy removal can alter stream temperature in other streams (e.g. Moore, Spittlehouse & Story, 2005), and water temperature can influence CR rates in streams (Bott *et al.*, 1985; Sinsabaugh, 1997; Uehlinger, Konig & Reichert, 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 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

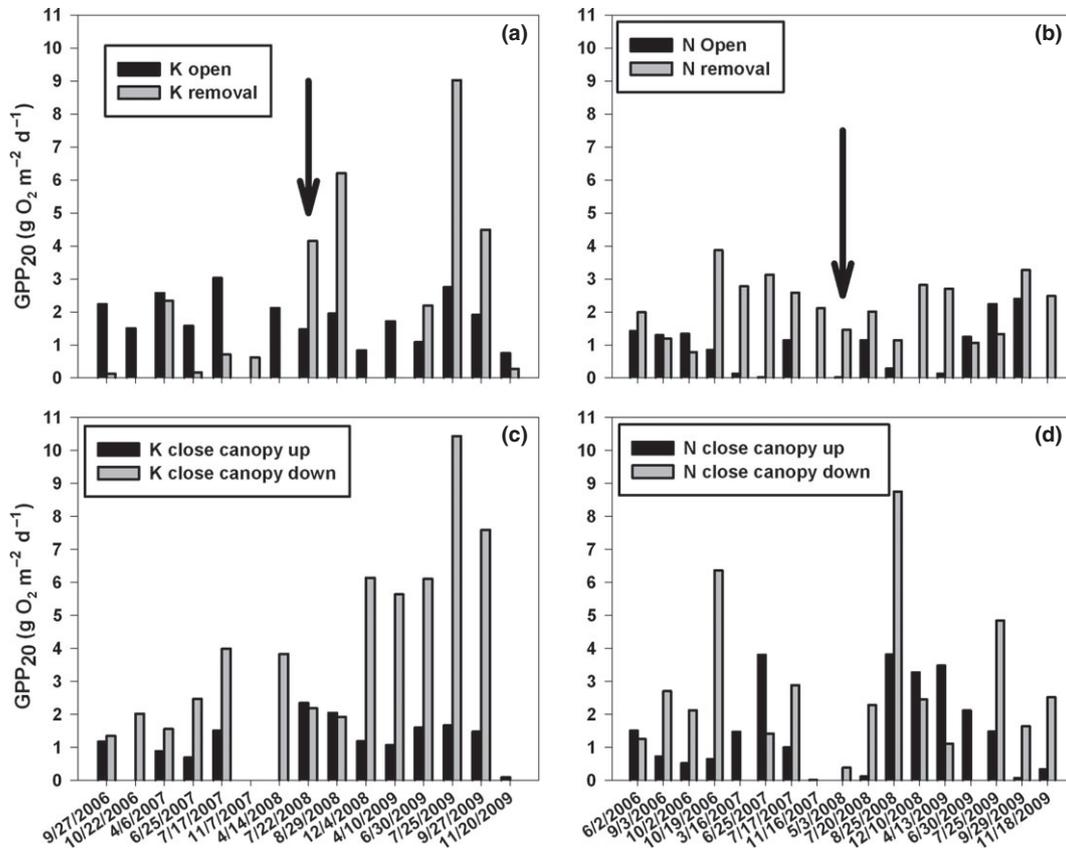


Fig. 5 GPP_{20} estimated during 2006–09 for all eight study reaches from subcatchments N04D (grazed) and K02A (ungrazed) in Kings Creek: Open and removal for (a) K02A and (b) N04D, (c) K02A closed-canopy reaches upstream and downstream from the removal reach and (d) N04D closed-canopy reaches upstream and downstream from the removal reach. The black arrow indicates the time of the first metabolism measurement in the removal reaches after vegetation removal occurred (December 2007). Reaches missing a rate for a sampling date (a zero bar) were attributable to equipment failure.

Table 4 ANCOVA results from two removal reaches (NR and KR) with GPP_{20} as the dependent variable, subcatchment as a categorical variable and days since flood and percentage canopy as continuous variables

	SS	d.f.	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
Percentage canopy	14.271	1	14.271	4.250	0.050*
Subcatchment	3.672	1	3.672	1.094	0.306
Error	80.584	24	3.358		

Significant results had a P -value ≤ 0.05 and are denoted by an asterisk (*). SS, sum of squared of error; d.f., degrees freedom; MS, mean sum square error; F, f statistic; P, significance.

stream or the amount of available sunlight. The eight reaches in Kings Creek were dominated by heterotrophic processes since NEP was almost always negative, regardless of canopy type. These findings accord with previous studies in Kings Creek that found this stream to be net heterotrophic, albeit not strongly so, even in areas with

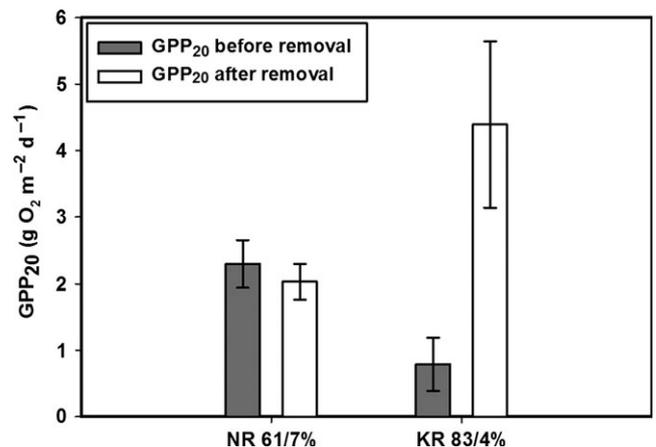


Fig. 6 Average GPP_{20} for just the removal reaches (NR and KR) in Kings Creek. The percentage canopy cover before/after the removal is displayed next to the reach code. Error bars represent standard error. Percentage canopy was significant with GPP_{20} (ANCOVA, $P = 0.050$). This was driven by the difference in the GPP_{20} before and after the removal for KR.

Table 5 ANCOVA results from eight reaches in Kings Creek for 2006 and 2007 (before riparian vegetation removal) with NEP as the dependent variable, season and subcatchment as categorical variables and days since flood, temperature and percentage canopy as continuous variables

	SS	d.f.	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
Percentage canopy	115.026	1	115.026	13.349	0.001*
Season	42.461	2	21.231	2.464	0.098
Subcatchment	3.929	1	3.929	0.456	0.504
Season*subcatchment	9.533	2	4.766	0.553	0.580
Error	336.064	39	8.617		

Significant results had a P -value <0.05 and are denoted by an asterisk (*). SS, sum of squared of error; d.f., degrees freedom; MS, mean sum square error; F, f statistic; P, significance; NEP, net ecosystem production.

Table 6 Average dry mass (DM) weight of filamentous algae collected during April, July and November/December of 2008 and 2009 for all eight reaches in Kings Creek with standard error in parentheses ($n = 3$)

Reach	Canopy (%)	Filamentous algae (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)

Open-canopy reaches (when open versus closed were compared) had greater amounts of filamentous algae than closed-canopy reaches (one-way ANOVA, $P = 0.006$). For meaning of acronyms, see Methods.

open canopy (Dodds *et al.*, 1996; Mulholland *et al.*, 2001; O'Brien & Dodds, 2010).

Before riparian vegetation removal, the greater the percentage canopy cover in Kings Creek, the greater the CR and the more NEP was pushed towards 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 entered the open-canopy reaches, and those that did were mostly removed. The organic matter from leaves increases microbial heterotrophic respiration (Roberts, Mulholland & Hill, 2007), so reaches with a greater amount of leaf litter could have greater heterotrophic respiration rates. An increase in heterotrophic respiration would increase total CR. The significant relationship

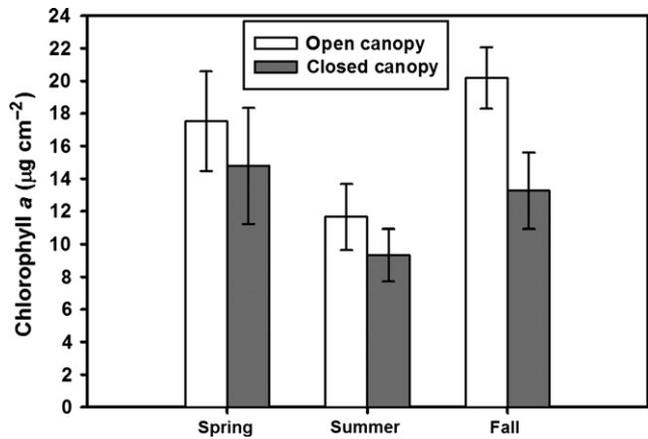


Fig. 7 Average chlorophyll *a* concentration for all reaches combined into open canopy (NO, NR, KO and KR) and closed canopy (NCU, NCD, KCU and KCD). Error bars represent standard error. Rocks were collected for chlorophyll *a* analysis in April, July and November/December of 2008 and 2009 (after removal). Open- and closed-canopy reaches differed marginally (factorial ANOVA, $P = 0.057$). Chlorophyll *a* results differed significantly between seasons (factorial ANOVA, $P = 0.031$).

between percentage canopy cover and CR before removal indicates a strong influence of allochthonous material in Kings Creek under current canopy conditions.

The hypothesis that GPP would be greater in open-canopy reaches in than closed-canopy reaches was supported for the removal reaches, but not across all eight reaches. When comparing just the removal reaches before and after vegetation removal, CR is low enough that the effects were not significant. However, the removal of vegetation in the removal reaches marginally significantly affected GPP₂₀.

There were minimal differences in primary production before and after vegetation removal for the grazed removal reach (NR). This is probably because of the orientation of NR and the south stream bank height, partially shading the stream in the afternoon. In the grazed subcatchment (N04D), the study area of Kings Creek has a greater sinuosity than in the ungrazed subcatchment (K02A), leading to increased shading of the stream in particular reaches. The significant relationship between canopy cover and GPP₂₀ for the removal reaches was driven by the difference in primary production for the ungrazed removal reach (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 the grazed removal reach (NR). Kings Creek flows in a more east-to-west direction in the ungrazed subcatchment (K02A) than in the grazed subcatchment (N04D), where it flows more south to north. Therefore, the ungrazed

removal reach (KR) could get more direct sunlight than the grazed removal reach (NR) driving higher GPP.

Greater GPP after vegetation removal agrees with the results of another study that found greater primary production in an open-canopy meadow stream than a closed-canopy forested stream (Bott, Newbold & Arscott, 2006). Other studies of small streams across land-use types and biomes have also found light to be a driving factor in whole-stream primary production (Mulholland *et al.*, 2001; Bernot *et al.*, 2010). An additional effect of increased sunlight associated with open-canopy reaches was also evident with greater amounts of filamentous algae in open-canopy reaches than in closed-canopy reaches.

Overall, the metabolism results indicate that light might not limit GPP as much as other factors in this system (e.g. nutrients). Nutrient bioassays have demonstrated algal biomass and heterotrophic microbial biomass in open areas of both reaches to be strongly limited by N and P (Johnson, Tank & Dodds, 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.

Variation of metabolism in prairie streams

Metabolism can vary seasonally (Wetzel, 2001). Individual GPP₂₀ rates from each reach were variable between seasons and among the same types of canopy cover, indicating seasonal effects in addition to temperature. We acknowledge that variability could simply be related to methodologically introduced variation, but some metabolic rates were statistically related to hypothesised drivers. Closed-canopy reaches within the same subcatchment often gave very different rates for measurements on the same day, indicating large spatial in addition to temporal variance. Thus, metabolism is probably dependent on reach-specific characteristics in addition to canopy cover. The maximum GPP₂₀ for reaches in Kings Creek tended to occur in the summer, but there were also high rates in the spring and autumn.

Higher primary production rates in spring may be the result of algal communities starting to develop (i.e. communities are more productive) as temperature and light increase from winter. In our study, spring chlorophyll concentrations were greater than those in summer. However, autumn chlorophyll concentrations were also greater than the summer values. Periphyton communities (including algae, cyanobacteria and heterotrophic bacteria) commonly peak in early spring or autumn (Gumtow, 1955; Cushing, 1967; Flemer, 1970; Marker, 1976). Mulholland *et al.* (2001) estimated 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 greater in open-canopy reaches than in closed-canopy reaches and significantly differed between 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 scouring from floods, as most floods in Kings Creek occur in late spring or early summer. Robinson & Minshall (1986) have documented seasonal differences in chlorophyll *a* in a mountain stream. They found greater chlorophyll *a* concentrations in autumn than in summer and greater concentrations in an open-canopy reach than in a closed-canopy reach. Seasonal changes in algal communities, and thus chlorophyll *a*, could contribute to seasonal trends in GPP₂₀ rates in Kings Creek.

In addition to variable GPP, CR was also highly variable across seasons and years. We hypothesised that seasonal changes in CR would be less pronounced after vegetation removal because of the decrease in leaf input to the reach. Temporal trends in CR did not support this hypothesis. Similar to the patterns of GPP, CR from the two closed-canopy reaches in both subcatchments were often very different for measurements from the same day, demonstrating substantial spatial variance in CR.

Metabolic rates from the eight reaches in Kings Creek, albeit variable, do fall within the range of metabolic rates from streams where metabolism is previously reported. Metabolism was estimated 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. 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 0.05–16.20 g O₂ m⁻² day⁻¹; Bernot *et al.*, 2010).

Our metabolism rates varied seasonally, and our results, particularly from closed-canopy reaches, are consistent with the study of Roberts *et al.* (2007). Our methods were designed to minimise day-to-day variation (sunny days at

baseflow), so we did not observe daily variation driven by light as reported by Roberts *et al.* (2007).

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

It is important to assess whether 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 towards CR rates estimated in the naturally open-canopy reaches. In the removal reaches after the removal, CR decreased after removal, bringing rates closer to those estimated in the naturally open reaches. Average GPP did not indicate that removal reaches match the naturally open reaches. Our study was only conducted for 2 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 removal. 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.

The endangerment of prairie streams makes continued research on these systems vital. Of the human activities impacting prairie streams, management practices that lead to encroachment by woody vegetation are yet another potential threat to an already rare ecosystem type. The unique aspect of prairie streams is open canopies in small headwater streams; therefore, it is important to preserve them if the desire is to retain native ecosystem functions downstream. 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 streams.

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