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Factors influencing periphyton growth in agricultural streams of central Illinois

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Abstract

Factors limiting periphyton accrual in east-central Illinois agricultural streams were investigated. Nutrient-diffusing substrata were used to examine periphyton macronutrient limitation in streams in two agricultural watersheds. Substrata consisted of sand-agar mixtures with one of six experimental treatments. Macronutrients included carbon, nitrate, phosphate and combinations of the three. Substrata were collected after a 5 and 9 day period and analyzed for chlorophyll *a*. None of the treatments were significantly greater than the controls at any of the seven stations, thus we conclude that periphyton in these streams was not nutrient limited. Highest periphyton colonization/growth rates were associated with the smaller upstream reaches, while lower rates occurred in the larger downstream reaches. Multiple regression showed that most of the variance in the rate of chlorophyll *a* accrual after five days was explained through water temperature and turbidity ($r^2 = 0.91$); whereas, stream nitrate and phosphate concentrations accounted for no significant portion of the variance. We conclude that instream primary production in agricultural streams of central Illinois is limited by temperature and light.

Introduction

Stream periphyton are known to be limited by an array of resources including light (McIntire *et al.*, 1964; Gregory, 1980; Lowe *et al.*, 1986), flow (Moore, 1977) and nutrients (Patrick, 1966; Pringle & Bowers, 1984). To date the majority of research on factors limiting stream periphyton has focussed on macronutrients, with methods including correlation techniques (Kilkus *et al.*, 1975), whole stream enrichment (Gregory, 1980; Elwood *et al.*, 1981), and the use of outdoor nutrient enhanced flumes (Stockner & Shortreed, 1978; Triska *et al.*, 1983). More recently, nutrient-diffusing substrata have been used in lentic (Fairchild & Lowe, 1984; Fairchild *et al.*, 1985) and lotic (Pringle & Bowers, 1984; Lowe *et al.*, 1986; Pringle *et al.*, 1986; Pringle, 1987) environments with apparent success. The advantages of a point source manipulation, such as nutrient-diffusing substrata, are: nutrient levels being released into a system can be controlled, other environmental variables (e.g. light, flow) can function normally, treatments can be replicated, and treatments can be interspersed within the stream.

The macronutrients nitrogen and phosphorus are commonly major limiting resources for periphyton in freshwater systems. Streams draining agricultural watersheds of the midwest charac-

teristically have elevated levels of both nitrogen and phosphorus, and highly variable N:P ratios (e.g. Kilkus *et al.*, 1975). Another important factor influencing periphyton communities in agricultural regions of the midwest is that low-order headwater reaches tend to have little canopy cover, whereas riparian canopy cover becomes more dominant in the larger downriver reaches. Wiley *et al.* (1987) found that primary production in an agriculturalized prairie river system ranged from below detection to over 50 g O_2 m^{-2} d^{-1}, with highest rates found in streams in the upper portions of the watershed. Correlative evidence suggests that algal productivity in these streams is not limited by either phosphorus or nitrogen (Kilkus *et al.*, 1975; Moore, 1977; Wiley *et al.*, 1987). However, to date there have been no experimental tests of macronutrient limitation in midwestern agricultural watersheds.

The objective of this study was to determine what factors most limited periphyton communities in agricultural streams of central Illinois. The first part of this study consisted of the experimental addition of nitrate, phosphate and carbon to assess whether periphyton is limited by the availability of macronutrients. A multiple regression model was then employed to determine factors contributing to periphyton accrual in these streams.

Description of study area

This study was conducted in the Salt Fork (SF) and Middle Fork (MF) branches of the Vermilion River located in east-central Illinois, USA (Figure 1). The Salt Fork and Middle Fork are adjacent to one another and encompass areas of approximately 1 300 and 1 100 square kilometers, respectively. Unlike many eastern and western North American streams (e.g. Vannote *et al.*, 1980), forested riparian vegetation is restricted to the lower portion of the mainstems of both rivers; riparian vegetation in headwater reaches generally consists of grass and small shrubs. Both watersheds drain clayey loess soils, and as a result receive most of their water from surface and shallow subsurface (tiled fields) runoff. The substratum in the upper portions of both rivers consists of coarse sand reflecting the low slope of the region. The lower reaches of both streams flow through a glacial moraine which increases slope resulting in a corresponding increase substratum particle size. Substratum in the lower reaches is dominated by small cobble and rubble in the riffle areas and sand and silt in pool areas.

The Salt and Middle Fork's of the Vermilion River flow through primarily agricultural regions, with 91% and 83% respectively, of their drainage in intensive row crop (corn and soybean) production (Osborne *et al.*, 1985). Despite the extensive agricultural nature of the two watersheds, high instream concentrations of nutrients (ie. NO_3-N and SRP), particularly during low-flow events,

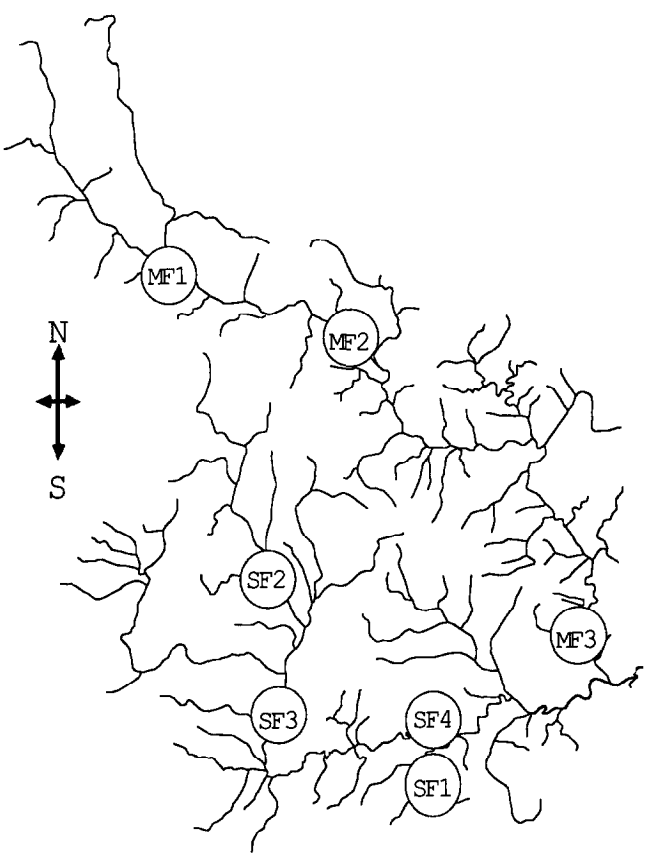

Fig. 1. Locations of the seven sampling stations on the Salt Fork (SF) and Middle Fork (MF) of the Vermilion River, Illinois.

can be largely attributed to the urbanization within the watersheds (Osborne and Wiley, 1988). The two basins differ in the extent of urbanization; the Salt Fork being roughly 5% urbanized and the Middle Fork roughly 1.1% urbanized. Despite the differences in absolute urbanization, the longitudinal distribution of urban landuse is similar in both watersheds with the highest proportion of urban areas occurring in the middle and upper reaches.

A total of seven sampling stations were established within the two watersheds (4 in the Salt Fork and 3 in the Middle Fork) to examine the hypothesis that instream primary production was not macronutrient limited (Figure 1). Sites were selected to provide a longitudinal comparison of periphyton communities and nutrient limitation based on station drainage area, stream order, extent of urbanization and extent of forestation.

Methods

Field experiment

Nutrient enriched sand-agar substrata were constructed following the method of Pringle & Bowers (1984). Medium sized sand (.295 to 1.0 mm) was washed and autoclaved to kill associated microorganisms. Approximately 40 g of sand was placed in a plastic petri dish (60 × 15 mm) with 10 ml of 2% agar containing one of six treatments. Nitrate and phosphate were mixed into the agar solution directly, autoclaved, and allowed to cool slightly before mixing with the sand; whereas, carbon was placed into each plate in dry form to avoid volatilization at high temperatures. All nutrient-diffusing substrata were allowed to dry before use. The six experimental treatments were as follows: (1) control (unenriched agar), (2) carbon (C) (1.0 M $NaHCO_3$), (3) carbon and nitrate (CN) (0.5 M $NaHCO_3$ + 0.5 M $NaNO_3$), (4) carbon, nitrate and phosphate (CNP) (0.5 M $NaHCO_3$ + 0.5 M $NaNO_3$ + 0.5 M KH_2P0_4), (5) phosphate (P) (1.0 M KH_2PO_4), (6) phosphate and carbon (PC) (0.5 M KH_2PO_4 + 0.5 M $NaHCO_3$).

One sample of each of the six treatments was glued onto a pine board in a random sequence. On 7 October 1987, six boards were placed at each of the seven stations in two sets of three; three boards were positioned end to end and perpendicular to the stream bank so that nutrients from one plate would not interfere with another. The downstream set was placed 30 to 50 meters from the upstream boards. We believe that the downstream boards were distant enough to minimize influences from upstream treatments. Boards were secured to utility bricks, setting them approximately 7 cm off the substratum. Boards were checked every few days to remove any debris that may have collected. Three replicates of each treatment were collected after 5 and 9 days from each of the seven stations. Instream nitrate (NO_3-N) and soluble reactive phosphorus (SRP) were determined at the start of the experiment using a Technicon Autoanalyzer. Physical parameters measured included turbidity (NTU's), bottom and surface light (ft. candles), water depth (cm), and water temperature (C).

To determine chlorophyll *a* the top 3 mm of the periphyton colonized sand-substratum was removed and mixed with 100 ml of distilled water. Algae were separated from the sand by mixing the solution with a magnetic stirrer for 10 minutes. Pringle & Bowers (1984) reported that this separation technique adequately obtained the majority of algal cells associated with sand particles. A 25 ml subsample was filtered onto a 0.2 μm Gelman glass fiber filter and analyzed for chlorophyll *a* using a Perkin-Elmer spectrophotometer (Model 124-D) (APHA, 1985). Intrasite differences in chlorophyll *a* concentrations attributable to nutrient treatments and time were examined using a one-way ANOVA (SAS, 1985).

An intersite comparison of instream periphyton accrual (production + colonization) was made using the daily rate of chlorophyll *a* increase (mg m^{-2} d^{-1}) on the control plates from each station based on the first five day period. An instantaneous accrual coefficient (r) was determined using the equation $N_t/N_o = e^{rt}$, where N_t is chlorophyll *a* (mg m^{-2}) for the first five day period, and N_o was arbitrarily set to 1. Accrual coefficients from control plates from each station

were incorporated into a multiple regression model as the dependent variable; independent variables included water temperature, turbidity, light coefficients (bottom light/surface light), water depth, NO_3-N and SRP.

Laboratory diffusion experiment

To determine the maximum diffusion rates of the three macronutrients from the sand-agar plates, three replicate plates were made with an equal combination of 0.5 M carbon, nitrate and phosphate (see treatment #4). Each plate was placed into a two liter beaker with 1.5 liters of distilled water. The three containers were placed in a water bath at 17 C with the water of each container continually mixed with a magnetic stirrer. Water samples were taken at 24, 48, 96, 144 and 216 hour periods and analyzed for alkalinity ($CaCO_3$), nitrate (NO_3-N) and soluble reactive phosphorus (SRP). After each sampling period the containers were refilled with fresh distilled water. The rate of diffusion of each macronutrient into the distilled water was determined by regression. These rates reflect maximum diffusion under laboratory conditions, therefore rates in the field will vary somewhat due to flow and other environmental conditions.

Results

Under laboratory conditions all three nutrients were released as a power function of time, with highest rates occurring during the first 24 hrs (Fig. 2). Carbon ($CaCO_3$) had the highest initial diffusion rate at 1390 μM d^{-1}, and ending with a rate of 35.3 μM d^{-1} after 216 hrs. Initial release rates of phosphate (SRP) were 645.9 μM d^{-1}, decreasing to 10.9 μM d^{-1} by 216 hrs. The most rapid diffusion was found with nitrate (NO_3-N) with an initial diffusion of 594.6 μM d^{-1} and dropping rapidly to 0.016 μM d^{-1} after 216 hrs.

In the field experiments there was distinct periphyton accrual by 5 and 9 days at all stations, with highest chlorophyll *a* values found at stations MF-1 and SF-1 for the 5 day period and at stations SF-1 and SF-2 for the 9 day period (Table 1). Some sand-agar plates were lost during the experiment due to falling water levels. We found no significant increases in chlorophyll attributable to nutrient treatments. A significant difference in chlorophyll *a* among treatments occurred at station SF-2 for the 5 day period (ANOVA, $p < 0.01$), with the control and PC treatments having significantly more chlorophyll than the C and P treatments (SNK test, $p < 0.05$).

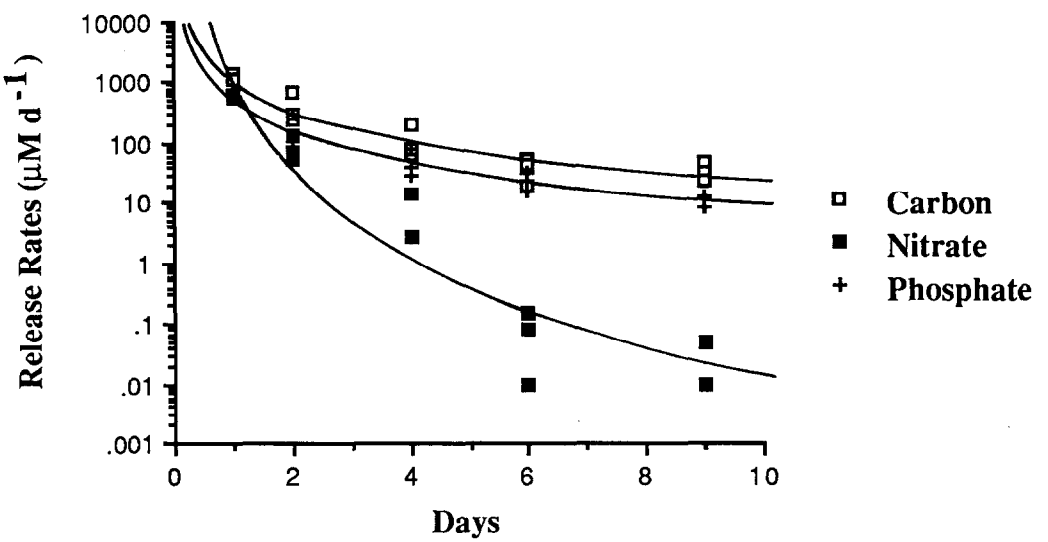

Fig. 2. Release rates of carbon, nitrate and phosphate under laboratory conditions. Carbon, $Y = 1197.0T^{-1.75}$, $r = 0.96$, $n = 15$. Nitrate, $Y = 1299.4T^{-5.04}$, $r = 0.96$, $n = 15$. Phosphate, $Y = 592.0T^{-1.84}$, $r = 0.97$, $n = 15$.

Table 1. Chlorophyll a values (mg m^2) for all stations and treatments. Values equal X ± SD (N). CONT = control, C = carbon, CN = carbon + nitrate, CNP = carbon + nitrate + phosphate, P = phosphate and PC = phosphate + carbon.

Station		Treatment					
		CONT	C	CN	CNP	P	PC
SF-1	5-day	7.0 ± 2.2 (3)	3.4 ± 2.2 (3)	16.7 ± 3.0 (3)	4.7 ± 1.4 (3)	5.4 ± 2.2 (3)	2.9 ± 2.5 (3)
	9-day	21.7 ± 9.5 (3)	8.0 ± 2.6 (3)	17.9 ± 5.3 (3)	7.3 ± 1.3 (3)	6.5 ± 4.9 (2)	17.6 ± 3.1 (3)
SF-2	5-day	4.8 ± 0.4 (3)	1.6 ± 1.1 (3)	4.4 (1)	3.0 ± 0.7 (3)	1.0 ± 0.9 (3)	3.9 ± 1.6 (3)
	9-day	26.6 ± 7.7 (3)	17.0 ± 9.5 (3)	22.8 ± 10.0 (3)	19.0 ± 2.1 (2)	27.0 ± 17.5 (3)	18.7 ± 11.0 (3)
SF-3	5-day	2.8 ± 2.1 (2)	2.7 ± 2.0 (3)	2.4 ± 0.3 (2)	1.1 ± 0.3 (2)	2.6 (1)	7.4 (1)
	9-day	1.7 (1)	5.7 (1)	1.7 (1)	2.6 (1)		0.0 (1)
SF-4	5-day	1.6 ± 0.6 (3)	1.0 ± 0.9 (3)	2.0 ± 1.1 (3)	4.5 ± 2.4 (3)	0.7 ± 0.9 (3)	2.0 ± 1.8 (3)
MF-1	5-day	7.5 ± 5.6 (3)	7.3 ± 4.9 (3)	7.4 ± 3.4 (3)	5.4 ± 4.4 (3)	0.9 ± 1.2 (2)	9.7 ± 6.4 (3)
MF-2	5-day	1.2 ± 0.9 (3)	0.7 ± 0.6 (3)	2.3 ± 0.9 (3)	1.7 ± 0.6 (2)	1.3 ± 1.2 (2)	1.3 ± 1.2 (2)
	9-day	2.6 ± 1.7 (3)	2.3 ± 1.6 (3)	3.2 ± 2.8 (3)	1.7 ± 1.6 (3)	2.7 ± 0.9 (3)	
MF-3	5-day	3.0 ± 2.8 (3)	3.4 ± 0.6 (3)	3.3 ± 1.5 (2)	1.9 ± 1.7 (3)	3.0 ± 1.8 (2)	2.0 ± 1.0 (3)
	9-day	15.4 ± 4.4 (3)	8.9 ± 2.8 (3)	10.8 ± 5.6 (3)	10.5 ± 5.8 (3)	9.8 ± 6.5 (2)	3.9 ± 9.6 (2)

A significant difference in chlorophyll *a* also occurred among treatments on day 9 at station SF-1 (ANOVA, $p < 0.05$); the control treatment was significantly greater than the C, CNP or P treatments (SNK test, $p < 0.05$). No other significant differences were found between any of the six treatments at any of the other five stations; nor was there any significant variation between the treatments when all the stations were combined. Grazers were rarely collected on the artificial substratum; when they were found they only consisted of a single chironomid larvae.

Periphyton accrual rates (mg chlorophyll a m^{-2} d^{-1}) at these seven stations were found to be negatively correlated with drainage area (Fig. 3). Accrual rates were highest in streams from smaller drainage areas and lowest in streams draining larger areas (Table 2). Stations located in larger reaches of the river system had slightly lower temperatures, but higher turbidity, and therefore less light penetration. There was little difference in rate of periphyton accrual between the Salt Fork and Middle Fork stations. Both watershed were relatively similar in relation to the independent variables measured except that nitrogen and phosphorus concentrations were slightly higher in the Salt Fork (Table 2). Further, the values of the independent variables were typical of previously reported values for these parameters within the two watersheds (Osborne *et al.*, 1985). Results from the multiple regression model showed that water temperature and turbidity accounted for the majority of variation in chlorophyll *a* accrual coefficients ($r^2 = 0.91$) (Table 3); while, the remaining independent variables accounted for no significant portion of the model.

Discussion

An important consideration in any *in situ* experiment is whether the technique used is sufficient to address the hypothesis being tested. In our study, the sand-agar plates released the nutrients in an exponential manner, with release rates decreasing rapidly during the first few days. Pringle & Bowers (1984) also found nitrate and phosphate released exponentially, with most of the nutrients released in the first 7 days. While all three of the nutrients we used were released rapidly, we believe that concentrations were sufficient to test

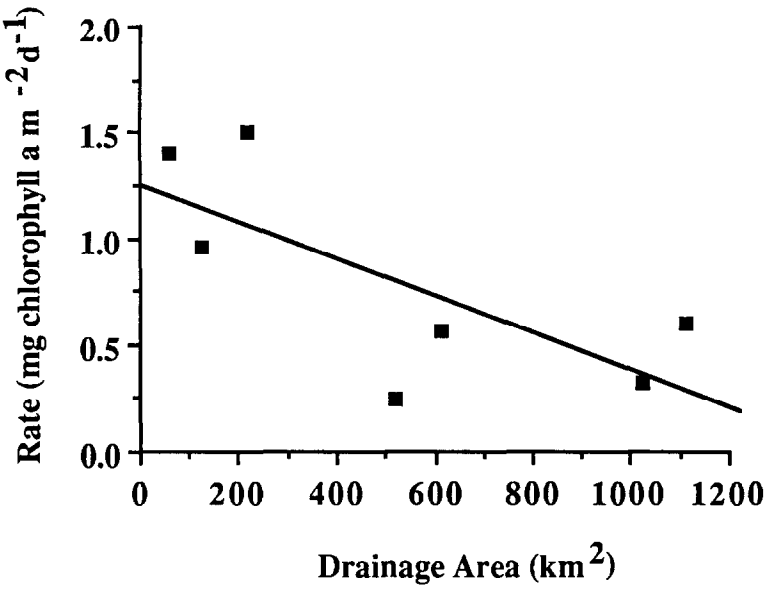

Fig. 3. Mean rate of chlorophyll *a* accrual against drainage area of station watersheds. Y = 1.25 – 0.001X, r = 0.73, n = 7.

for macronutrient limitation. Release rates of nutrients into the water were high, 594–1390 μM d^{-1}, rates three to six times higher than rates reported by Pringle & Bowers (1984) for 0.5 μM substrata at 10 C. The substrata used in our field experiments contained as much as 1 mole of nutrient in single nutrient treatments, therefore nutrient release rates would be much greater for these treatments.

The significant difference among treatments on a particular date is difficult to explain since it was the control and PC treatment (station SF-2) and control (station SF-1) which were significantly higher than the other treatments. Given the number of statistical comparisons (14), it would not be surprising to have at least one significant difference due to chance alone. It is possible that some factors in the stream reduced the ability for periphyton to grow (e.g. detritus accumulation) or removed periphyton which had already colonized substrata (e.g. grazers). Our periodic checking and clearing of any accumulated debris and the

Table 2. Chlorophyll *a* accrual coefficients and mean rates (mg chlorophyll *a* m^{-2} d^{-1}) along with independent variables used in the multiple regression model. Independent variables were collected on October 8, 1986.

Stream		Drainage Area	Accrual	Independent Variables					
Station	Order	(km^2)	Coeff. (Rate)	Temp (C)	Turb (NTU)	Light Coeff.	Depth (cm)	NO_3 (mg l^{-1})	SRP (mg l^{-1})
MF-1	2	85.3	0.40 (1.50)	22.5	4.8	0.53	12.2	1.8	0.33
MF-2	3	200.0	0.04 (0.24)	16.0	14.6	0.27	29.0	1.8	0.85
MF-3	5	429.9	0.22 (0.60)	18.0	10.3	0.21	40.2	2.1	0.52
SF-1	2	24.0	0.39 (1.40)	18.5	3.5	0.37	32.0	4.0	2.40
SF-2	3	48.3	0.31 (0.96)	18.5	2.0	0.50	22.0	4.2	2.50
SF-3	4	236.5	0.21 (0.56)	15.5	4.8	0.29	38.7	5.8	2.90
SF-4	5	359.0	0.09 (0.32)	16.5	19.0	0.12	36.0	2.0	0.80

Table 3. Summary of ANOVA for multiple regression model. Y = −0.174 + 0.013 (TEMP) − 0.013 (TURB), R^2 = 0.91.

Dependent variable: Rate					
Source	*DF*	Sum of *Square*	Mean *Square*	*F Value* *P*	
Model	2	0.106	0.053	19.36	0.009
Error	4	0.011	0.003		
Corrected Total	6	0.117			

R-Square	*CV*	*Root MSE*	*Rate Mean*
0.01	22.04	0.052	0.237

Parameter	*Estimate*	*T for Parameter*	*P*
Intercept	−0.174	−0.87	0.433
Temperature	0.030	2.87	0.045
Turbidity	−0.013	−3.60	0.004

very limited number of grazers encountered on the substrata minimizes the probability of these variables affecting the results. The lack of a significant difference between all treatments when all stations were combined demonstrates that the control plates did not have some unique property that enhanced algal colonization and/or growth over the other treatments.

Regardless of the above two anomalies, periphyton in these streams do not respond to point sources of the three nutrients examined in this study. These findings are consistent with the observation that nitrate and phosphate levels in the Vermilion River are extremely high, and support earlier correlative studies (Wiley *et al.*, 1987). Previous studies on agricultural streams have also found a lack of correlation between periphyton standing crops and increases in either nitrate or phosphate (Kilkus *et al.*, 1975; Moore, 1977; Patrick, 1966). Kilkus *et al.* (1975) examined agricultural streams in Iowa and concluded that in-stream nitrate (NO_3-N) and phosphate (PO_4-P) levels were so high, 1.75 mg l^{-1} and 0.16 mg l^{-1} respectively, that they could not detect an increase of algal biomass with higher levels of nutrients. In the Salt Fork and Middle Fork Rivers NO_3-N levels range from 0.0 to 9.2 mg l^{-1}, and SRP from 0.0 to 9.7 over an annual period (Wiley and Osborne, unpubl. data), exceeding those found in the Iowa study.

While macronutrient limitation was not found in our streams, other studies have reported nitrogen and phosphorus limitation in nonagricultural streams. Nitrogen limitation was reported from an artificial stream (Triska *et al.*, 1983) and a natural forested stream (Gregory, 1980) in the western United States when light levels were increased; whereas, Grimm *et al.* (1981) suspected nitrogen limitation in a desert stream in the southwest where light levels are sufficient. In contrast, Peterson *et al.* (1983) and Stockner & Shortreed (1978) found streams in the Pacific Northwest to respond to additions of phosphorus or phosphorus + nitrogen, but not to nitrogen alone. Studies from eastern US regions also report a variety of results ranging from nitrogen and nitrogen + phosphorus limitation (Crawford, 1979) to phosphorus limitation (Elwood *et al.*, 1981; Pringle & Bowers, 1984) in woodland streams.

Whereas many studies have addressed nitrogen and phosphorus limitation, few have assessed carbon limitation in streams. Carbon is rarely studied since some believe that it can not become limiting in streams due to the complex carbon cycle involving atmospheric CO_2 (Peterson *et al.*, 1983). While carbon limitation may be rare, Dickman (1973) and Crawford (1979) both demonstrated an increase in algal standing crop after the addition of HCO_3^-. King (1970) states

that carbon may become limiting when nitrate and phosphate levels are high, as was found in the Madison River (Wright & Mills, 1967). Although periphyton in our study did not respond to inorganic carbon, it is possible that carbon may become limiting under specific conditions. For example, when flow rates are very low during the summer, rates of gross primary productivity can reach 50 g O_2 m^{-2} d^{-1} at some sites in the Vermilion River (Wiley *et al.* 1987). Under these conditions inorganic carbon demand could exceed the supply during specific times of the day.

Our study did not address several issues which are undoubtedly important to understanding algal community dynamics in agricultural streams. When macronutrients are found in sufficient quantities, as was the case in our study, micronutrients may become limiting. For example, Pringle *et al.* (1986) examined a stream in Costa Rica and found that additions of nitrate and phosphate alone failed to show an increase in periphyton, whereas a micronutrient combination (Fe, B, Mn, Zn, Co, Mo, EDTA) supplemented with and without nitrate and phosphate, elicited a positive response.

While our study has focussed on a community response to nutrient enrichment, algal species may respond differentially to resource conditions. Pringle & Bowers (1984) and Lowe *et al.* (1986) reported a differential response of algal species on the various nutrient treatments. The limiting factor for a specific algal species can also vary throughout the year. Zevernboom *et al.* (1982) reported that phosphorus, nitrate, light and temperature were all limiting to the growth rate of *Oscillatoria agardhii* at different times of the year. Thus, while the algal community as a whole did not respond to nutrient additions in our experiment, individual species may have been able to capitalize on a specific treatment.

While we did not detect a treatment effect at the seven sampling stations, we did find that chlorophyll *a* accrual rates were relatively high for the first five day period ($\overline{X}$ = 0.80 mg chlorophyll *a* m^{-2} d^{-1}). This is comparable to the 1.1 mg chlorophyll *a* m^{-2} d^{-1} reported by Liaw & MacCrimmon (1978) in a river draining an agricultural region of southern Ontario, but greater than that reported from nonagricultural streams in Michigan (0.52 mg chlorophyll *a* m^{-2} d^{-1}) (Meier *et al.*, 1983) and Costa Rica (0.48 mg m^{-2} d^{-1}) (Pringle *et al.*, 1986). These high rates of chlorophyll *a* accrual are in themselves supportive of a hypothesis of no nutrient limitation.

To better assess factors limiting instream primary production, we examined longitudinal patterns in periphyton accrual and correlations with environmental factors. The significant relationship between chlorophyll *a* rates and drainage area indicates that smaller upstream reaches were generally more productive than larger downstream reaches. This longitudinal pattern is best explained by the multiple linear regression results (Table 3) which showed that water temperature and turbidity account for the majority of variation in chlorophyll *a* accrual rates. The upstream stations have shallow open channels with higher water temperatures and more light. As one moves downstream channels are bordered by gallery forests resulting in lower water temperatures and increased shading. While temperature is not considered a resource, it does interact with other variables to enhance primary production. Kilkus *et al.* (1975) reported that water temperature was a major driving variable for periphyton in agricultural streams in Iowa. The importance of turbidity to instream primary production in our streams can not be overestimated since it was the most highly correlated variable with chlorophyll *a* accrual. Turbidity was low in the smaller upstream reaches and therefore the periphyton communities had higher light levels for growth. In contrast, downstream reaches become much more turbid due to hydraulic and edaphic characteristics. With increased turbidity there is a concurrent reduction in light penetration. Light is known to be an essential resource for stream periphyton communities (Gregory, 1980; McIntire *et al.*, 1964) and in agricultural regions like Illinois, may be the dominant limiting factor.

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