In situ nutrient enrichment experiments with periphyton in agricultural streams[1]

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Abstract

Four agricultural streams were examined to determine if nitrogen or phosphorus was limiting the growth of the attached algal communities. Experiments were conducted in situ using nutrient-diffusing artificial substrata. Experiments conducted over a 2-year period demonstrated that the benthic algal communities in these streams were seldom limited by nitrogen or phosphorus. Nitrogen was, however, found limiting on two occasions when ambient nitrogen levels were reduced. These experiments were characterized by extended low flows and warm water temperatures. Large algal mats which proliferate during these periods may be responsible for reducing the available nitrogen to a limiting level. Major storm events are thought to interrupt the development of nitrogen limiting conditions by scouring the algae and increasing the nitrogen loading from the watershed. Water temperature was demonstrated as an important factor in controlling periphyton growth rates and may have influenced algal response to nutrient input. Ammonium additions often enhanced algal growth in the absence of nitrate stimulation.

Introduction

Many recent advances in our understanding of small streams are based on studies in relatively undisturbed, forested ecosystems. Most of these streams have low nutrient concentrations and are heavily shaded from solar radiation, so that autotrophic production within streams is small and of little importance. These streams differ from those found in heavily cultivated regions. In Iowa (USA), for example, watersheds that were origi-nally grasslands have been converted to intensive row-crop agriculture. It is common for cultivation to take place up to the stream banks, so there are few if any trees to shade the water surface. A combination of rich prairie soils, heavy fertilizer usage and livestock production contributes to high concentrations of phosphorus and nitrogen in these waters (Omernick, 1977; Hill, 1978; Klepper, 1978: Neilsen *et al.*, 1982). In contrast to streams in forested areas, these streams contain high densities of benthic and planktonic algae.

Previous studies on agricultural streams have demonstrated that attached algal communities are greater in biomass and indeed the source of suspended algae (Swanson & Bachmann, 1976). Kortge (1984) found that the biochemical activities associated with the benthic algae could drastically affect water quality in small agricultural streams. In general, most nutrient/algae research has focused on suspended algae, not periphyton.

The primary objective of this study was to examine the relationship between nutrient availability and periphyton growth in agricultural streams by conducting a series of in situ nutrient enrichment experiments. Results from experiments conducted on four streams simultaneously over a two-year period are presented.

Materials and methods

Substrata preparation

In situ bioassays were conducted with nutrient diffusing artificial substrata similar to Fairchild & Lowe (1984). Unglazed clay flower pots (O.D. = 8 cm; height = 8.8 cm) were soaked in distilled water 72 h. Pot bottoms were completely sealed with a cork stopper and silicone sealant. A 4% agar solution (Difco Bacto Agar) either enriched with a potentially limiting nutrient (treatment) or without nutrients (control) was poured into each pot. After the agar solidified, a standard 100 × 15 mm plastic petri dish was fitted over the large opening and sealed tight with silicone. Nutrients diffuse through the porous clay walls, becoming available to the periphyton community growing on the outside.

Nutrient leaching rates were estimated in the laboratory by placing experimental pots into covered, acid-washed aquaria with 3.5 L of distilled water. A mild current was generated by bubbling air into the water. Water samples were collected in acid-washed polyethylene bottles and flower pots placed into clean aquaria every 24 h. Samples were frozen for later analysis. This technique was also used to determine the best substrata preparation technique (e.g. agar density, nutrient concentration, % pot surface area sealed with silicone) to obtain high, prolonged, leaching rates.

In situ experiments

In situ experiments consisted of five substrata types; one control and four nutrient treatments (Table 1). The first experiment included a comparison of two ammonium treatments of different concentrations. Experiments were conducted simultaneously on four streams within a 25 km radius in central Iowa, USA. Streams were representative of agricultural streams in the region. Stream characteristics are presented in Table 2. Experimental substrata were located at three sites within each stream. The sites were chosen to be as similar as possible with respect to current velocity, water depth, stream orientation and riparian shading (all sites had minimal shading). Sites were located 40–100 m apart. At each site, two fence posts were driven into the streambed to hold a 1.5 m, 2 × 4 cm wooden board in place perpendicular to the current. Five pots, one of each

Table 1. Artificial substrata treatments and corresponding nutrient concentrations.

Treatment	Concentration
Control (C)	4% agar solution
Nitrate (N)	2.5 M $NaNO_3$ + agar
Ammonium (Am)	0.5 M NH_4Cl + agar
Phosphorus (P)	1.0 M KH_2PO_4 + agar
Am + P (AmP) (Experiments 2–9)	0.5 M NH_4Cl + 1.0 M KH_2PO_4 + agar
Ammonium (Amx) (Experiment 1 only)	1.0 M NH_4Cl + agar

Table 2. Stream Characteristics. (Order = stream order, width = average stream width at study sites, W.A. = upstream watershed area, Crops = % of W.A. in row crops).

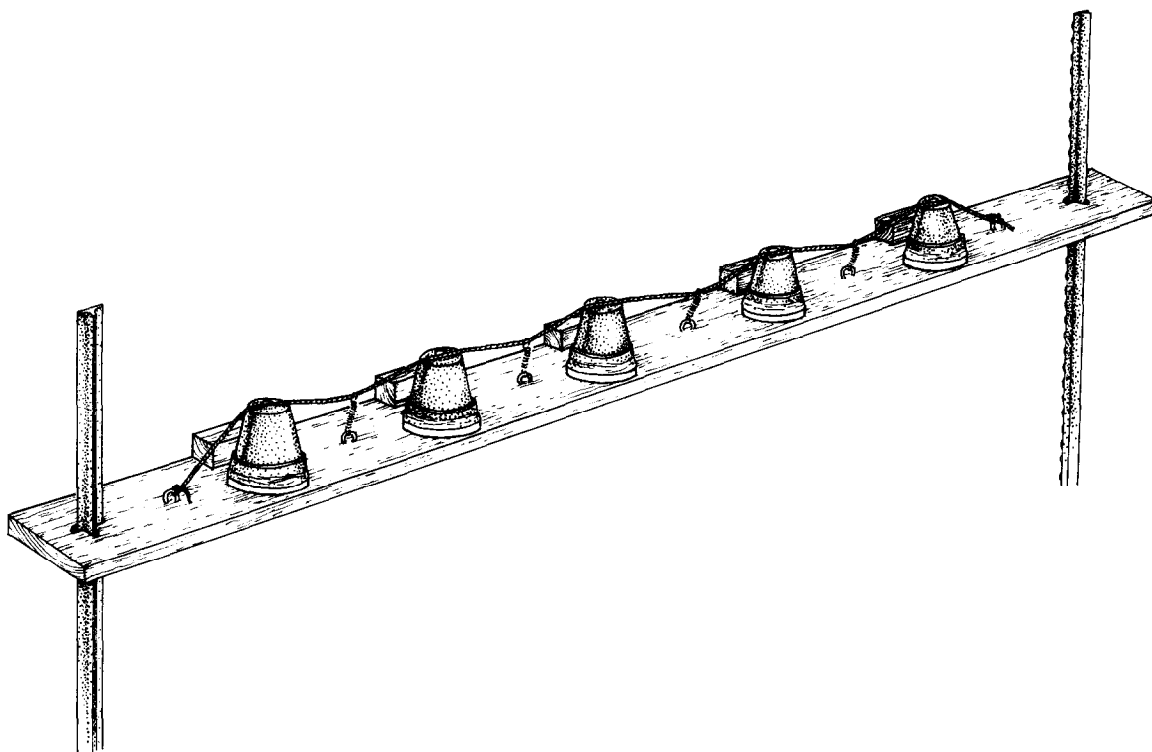| Stream	Order	Width	W.A.	Crops
		m	km^2	% W.A.
Big Cr.	2	2–3	18	84
Keigley Cr.	3	4–6	122	88
Squaw Cr.	4	12–14	530	84
South Skunk R.	4	16–20	819	89

Fig. 1. Diagram of board design used to secure substrata in the stream.

treatment, were placed in random order on each board. Pots were secured to the board with a rope threaded through springs located between each pot (Fig. 1). Pots were spaced 15 cm apart on the board to minimize the effect of water currents created by adjacent pots. The posts maintained a permanent site in the stream that could withstand scouring and shifting substrates while the height of the board could be adjusted on the posts to accommodate changes in stream stage.

Experiments were conducted during June–November 1984, and May–August 1985. Algal accumulation rates were rapid, therefore test durations were relatively short, 7–14 d, to minimize sloughing of cells. At the end of each experiment, pots were removed and sampled according to Fairchild *et al.* (1985). The 125 cm² of surface area above the enlarged lip and below the sealed circular top was scraped with a firm-bristle toothbrush and knife blade to remove attached algae. The scrapings and rinse water were collected in 500-ml neoprene bottles and immediately placed in the dark on ice for transport. All pots

were shaded during sampling to avoid exposure to direct sunlight.

In the laboratory, algal subsamples were filtered through Whatman glass fiber filters (1.2 μm nominal porosity) and frozen in a container with desiccant for later chlorophyll *a* analyses. Pigments were extracted with dimethyl sulfoxide and 90% acetone (Shoaf & Lium, 1976). Samples were extracted for 20 h, shaken and centrifuged. Optical densities were determined with a Beckman DU-2 spectrophotometer by using the trichromatic method and equations of Strickland & Parsons (1968), corrected for phaeopigments (Wetzel & Likens, 1979). Chlorophyll a values were expressed as mg m^{-2} of substrata area.

Measurements and analyses of stream water

Chemical and physical parameters were measured in each stream with samples taken three times during the course of each experiment. Du-

plicate water samples for nitrate and ammonium analyses were collected in acid-washed polyethylene bottles above the most upstream site. Samples were placed immediately on ice with nitrate samples first acidified with concentrated H_2SO_4 (1 ml l^{-1}). Duplicate 50-ml samples were collected for total phosphorus in acid-washed 125-ml Erlenmeyer flasks. Ammonium and nitrate samples were filtered through Whatman glass fiber filters (1.2 μm nominal porosity) upon returning to the laboratory. Ammonium analyses were conducted immediately after filtering using the colorimetric phenate method (APHA, 1981). Nitrate-nitrite concentrations were measured by cadmium reduction (Wetzel & Likens, 1979) after samples were first neutralized with NaOH. Total phosphorus samples were stored at 8–12 °C and analyzed using the method described by Murphy & Riley (1962) after a persulfate oxidation (Menzel & Corwin, 1965). Spatial comparisons of nutrient levels in the streams confirmed that nutrients released from upstream sites did not affect water quality at downstream sites. In addition to nutrients, the following parameters were evaluated: temperature (°C), pH, turbidity (NTU), specific conductance (μmhos cm^{-1}), discharge (m^3 s^{-1}), current velocity (m s^{-1}) and depth (m). Each site was checked every other day to adjust boards to stream stage. Boards were maintained at equal depths between sites with flower pots typically 12–15 cm below the surface.

Statistical analyses

Analysis of variance (ANOVA) was used to test for significant treatment effects in each experiment by pooling data from all four streams. Sources of variation in the model statement of each experiment ANOVA included: STREAM, SITE(STREAM), TREATMENT and STREAM*TREATMENT interaction. The Least Significant Difference test (LSD) was used to distinguish differences among treatments (alpha = 0.05). By conducting a pooled ANOVA the statistical leverage was increased for each experiment (i.e. n = 60). Although treatment effects which might have occurred in an individual stream may be obscured, overall trends among similar agricultural streams can be better evaluated. A detailed examination of every experiment conducted on each stream can be reviewed in Bushong (1985). ANOVA was also performed on data pooled from all experiments to evaluate important interactions.

Results

Nutrient diffusion rates

Laboratory experiments lasted 14 d at 21 °C ($\pm$ 1 °C). All treatment substrata leached nutrients throughout the 2-week period. The nitrate diffusion rate decreased 55% over fourteen days from an initial high of 155 mg N d^{-1}. The low and high ammonium treatments demonstrated comparable declines in leaching rates (approximately 32%) from initial peaks of 68 and 186 mg N d^{-1}, respectively. After an initial pulse, the phosphorus diffusion rate gradually increased to a high of 155 mg P d^{-1} day 11 before beginning to decline. Fairchild et al. (1985) found a similar initial increase in phosphorus diffusion rate. No nitrogen and only trace levels of phosphorus were detected leaching from control substrata. By increasing nutrient concentrations and agar densities within the flowerpots, and by decreasing the surface area available for leaching, relatively prolonged, high leaching rates were obtained.

In situ bioassays

A total of 14 experiments were conducted, seven in each of 1984 and 1985. Heavy rains and flooding decreased the number of useable experiments to nine, six in 1984 and three in 1985. Four experiments had slightly less than the full 60 observations (57–59) and the last experiment was conducted on only three streams because Keigley Creek dried. ANOVA demonstrated that differences among treatment chlorophyll a levels in experiments 1–4 and 8–9 were highly significant (P = 0.0001; except experiment #2, P = 0.01). No significant treatment effects were observed in experiments 5–7 (P $\geq$ 0.34). Table 3 shows the mean chlorophyll a levels pooled over streams for each treatment during the nine experiments.

Table 3. Treatment chlorophyll *a* values for each experiment. Rank was determined by the LSD test (P < 0.05). Treatments with no letter in common are significantly different within an experiment.

	Trt	Chl *a* (mg m^{-2})	Rank		Trt	Chl *a* (mg m^{-2})	Rank
Experiment	C	22.07	B	Experiment	C	3.49	A
#1	P	22.77	B	#6	P	3.42	A
7/31–8/10	Am	31.37	A	11/13–11/27	Am	3.40	A
1984	N	23.66	B	1984	N	3.73	A
	Amx	16.40	C		AmP	4.88	A
Experiment	C	5.86	B	Experiment	C	36.11	A
#2	P	6.00	B	#7	P	31.76	A
9/6–9/13	Am	8.19	A	5/23–6/3	Am	40.15	A
1984	N	6.45	B	1985	N	37.38	A
	AmP	6.27	B		AmP	35.04	A
Experiment	C	4.31	C	Experiment	C	28.72	B
#3	P	4.65	C	#8	P	27.81	B
9/20–9/27	Am	7.31	B	7/3–7/11	Am	41.12	A
1984	N	5.56	C	1985	N	29.52	B
	AmP	9.78	A		AmP	43.28	A
Experiment	C	5.61	B	Experiment	C	10.46	C
#4	P	5.06	B	#9	P	11.85	C
10/4–10/16	Am	9.74	A	8/1–8/10	Am	23.52	A
1984	N	11.07	A	1985	N	17.76	B
	AmP	11.05	A		AmP	21.31	A
Experiment	C	3.62	A				
#5	P	3.21	A				
10/23–11/6	Am	3.13	A				
1984	N	4.08	A				
	AmP	5.07	A				

Phosphorus treatments were never significantly different from controls. Low level ammonium treatments significantly enhanced chlorophyll *a* values in experiments 1–4 and 8–9 whereas nitrate additions demonstrated significantly higher values in experiments 4 and 9 only. The high level ammonium treatment 'Amx' had significantly lower chlorophyll *a* values than the control. Treatment 'AmP' enhanced chlorophyll *a* values in experiments 3–4 and 8–9. Experiments 5–7 demonstrated no treatment effects.

Stream effect was significant in each experiment (P < 0.01), however, algal response to treatments were generally comparable between streams within an experiment (STREAM*TREATMENT*EXPERIMENT interaction; P = 0.89).

Colonization rates were not taken into account when analyzing the chlorophyll *a* data. It was assumed that colonization rates were equal between substrata within a stream for any experiment. Although the percent of measured chlorophyll *a* which can be attributed to growth on the substrata is unknown, the analyses indicate that enough growth occurred during the experiments to indicate treatment response.

Table 4. Selected stream water quality parameters during nine experiments. Values are averaged over three sampling periods. (Kg = Keigley Cr., Sk = S. Skunk R., Sq = Squaw Cr. and Bg = Big Cr.).

Str	Exp	Temp	Discharge	Total-P	NO_3-N	NH_3-N
		(°C)	($m^3 s^{-1}$)	($mg\, l^{-1}$)	($mg\, l^{-1}$)	($mg\, l^{-1}$)
Kg	1	23.0	0.54	0.08	7.82	0.24
Kg	2	17.0	0.03	0.07	0.55	0.04
Kg	3	12.7	0.02	0.09	0.16	0.04
Kg	4	11.8	0.02	0.05	0.12	0.05
Kg	5	5.3	0.14	0.11	4.73	0.09
Kg	6	2.7	0.21	0.07	9.10	0.13
Kg	7	16.0	0.19	0.05	7.72	0.13
Kg	8	22.5	0.04	0.10	4.22	0.10
Sk	1	23.0	105.70	0.16	7.31	0.21
Sk	2	16.3	12.70	0.19	1.32	0.45
Sk	3	14.8	16.20	0.13	0.44	0.18
Sk	4	13.2	24.50	0.17	0.40	0.16
Sk	5	6.3	26.70	0.33	3.74	0.08
Sk	6	3.3	44.30	0.26	7.18	0.15
Sk	7	17.3	47.00	0.18	7.74	0.11
Sk	8	24.3	9.80	0.20	3.81	0.15
Sk	9	21.5	2.50	0.22	0.97	0.26
Sq	1	27.3	47.30	0.09	6.71	0.13
Sq	2	18.3	2.67	0.11	0.24	0.06
Sq	3	13.0	1.80	0.15	0.29	0.17
Sq	4	12.2	1.90	0.07	0.15	0.23
Sq	5	6.3	12.80	0.14	2.23	0.10
Sq	6	4.5	44.30	0.15	5.21	0.13
Sq	7	16.8	20.30	0.08	6.37	0.09
Sq	8	24.7	9.20	0.10	3.44	0.12
Sq	9	21.5	2.30	0.11	1.27	0.09
Bg	1	22.2	0.03	0.11	7.46	0.12
Bg	2	15.9	0.01	0.20	0.55	0.15
Bg	3	13.3	0.03	0.25	1.15	0.04
Bg	4	12.2	0.01	0.12	0.65	0.03
Bg	5	9.0	0.03	0.15	5.52	0.03
Bg	6	7.0	0.04	0.12	7.70	0.02
Bg	7	19.3	0.05	0.12	8.00	0.25
Bg	8	19.3	0.11	0.11	10.64	0.09
Bg	9	19.7	0.05	0.18	5.55	0.06

Water quality

Measured chemical and physical parameters are presented for each stream and experiment in Table 4. Values represent the mean of three sampling periods. Many of the variables were similar between streams. Water temperatures were similar and dropped sharply prior to experiments 5 and 6 in every stream. Although discharge differed between streams due to size differences, Keigley Creek, Squaw Creek and South Skunk River exhibited similar trends. In general, discharge in 1984 decreased rapidly after experiment 1 and remained relatively low until increasing in experiments 5 and 6. In 1985, dis-

charge decreased after experiment 7, becoming lowest during experiment 9. In contrast, discharge in Big Creek fluctuated without following a seasonal trend.

Nitrate concentrations were correlated with discharge for each stream. In 1984, nitrate levels were lowest during experiments 3 and 4. Measured concentrations from individual sampling periods were reduced to 0.066, 0.089, 0.335 and 0.384 mg l⁻¹ NO₃-N for Keigley Creek, Squaw Creek, Skunk River and Big Creek, respectively. In 1985, nitrate levels were lowest during and just preceeding experiment 9. Ammonium-N and total-P levels tended to fluctuate between experiments with no noticable trend. Turbidity, pH and conductivity were similar between streams with ranges of 1.8–22.3 NTU, 7.5–8.5 and 413–983 μmhos cm⁻¹, respectively.

The relationship between algal growth rates and in situ chemical or physical parameters was evaluated using data from the controls. The data were normalized to account for the effect of experiment duration by dividing chlorophyll a data by the length of the experiment in days to approximate accumulation rates. The most convincing relationship was the positive correlation between water temperature and biomass accumulation rate (R = 0.63; P = 0.0001), (Fig. 2). A complicating factor involved with this correlation is that in experiments characterized by colder water temperatures the photoperiod was reduced (i.e. experiments 5 and 6).

Discussion

The agricultural streams evaluated in this study generally contained high concentrations of nitrogen and phosphorus. Phosphorus enrichment never stimulated periphyton growth. Nitrogen in both forms (ammonium and nitrate) only enhanced growth in two experiments. The enrichment experiments therefore indicate that nitrogen and phosphorus levels are typically sufficient for algal demands and are therefore not limiting. These findings are consistent with previous studies which examined suspended algae in agricultural
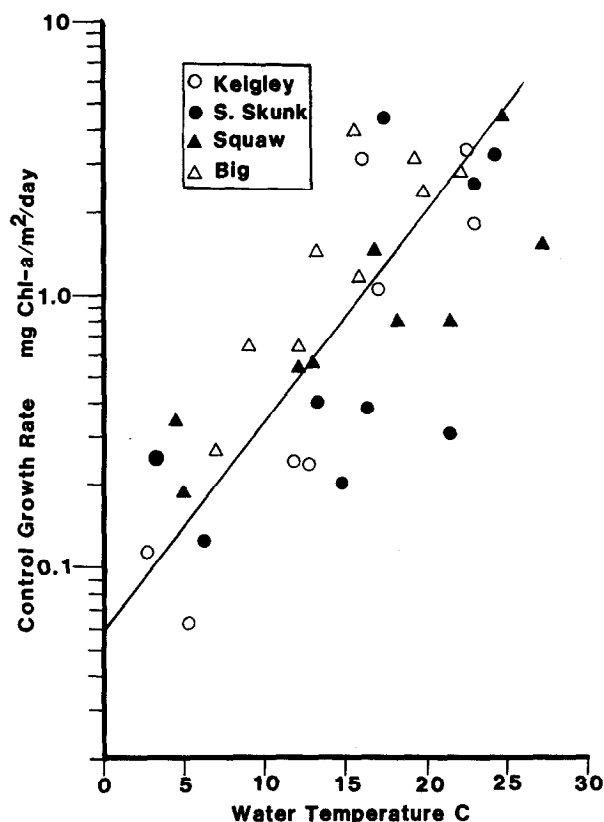

Fig. 2. Relationship between water temperature and algal growth rate on control substrata (R = 0.63, p = 0.0001). Each symbol represents the mean of three control substrata and mean water temperature during each experiment.

streams (Kilkus *et al.*, 1975; Burkholder-Crecco & Bachmann, 1979).

In four experiments, ammonium additions but not nitrate stimulated growth. This response was not interpreted as nitrogen limitation since both forms would have contained the limiting element. Less energy is required to assimilate ammonium than nitrate because ammonium is available for direct utilization after uptake. Nitrate, in contrast, must first be reduced to ammonium before utilization, a process that requires substantial energy (Syrett, 1962; Morris, 1974). Algal preference for ammonium, as a nitrogen source has been well documented in laboratory cultures and field studies (McCarthy *et al.*, 1977; Liao & Lean, 1978: Ward & Wetzel, 1980a, 1980b: McCarthy *et al.*, 1982). Syrett (1962) stated that stimulation

of growth by ammonium and not nitrate additions could indicate energy limitation (i.e. the energy conserved through ammonium utilization becomes available for growth). Energy limiting conditions could potentially occur if the peripheral cells of the periphyton community shade the cells within the algal mat. Hoagland et al. (1982) suggested that such vertical gradients exist within a periphyton community for factors such as light and nutrients.

Compared to studies on phytoplankton, the ambient ammonium levels found in this study would appear sufficient to inhibit nitrate assimilation by suppressing the enzyme nitrate reductase (Eppley et al., 1969; McCarthy, 1980). It seems unlikely that this occurred, however, since nitrate stimulated growth in two experiments. The apparent disagreement could be due to differences in algal species. The presence of a vertical gradient in nutrient concentrations could also explain this by essentially reducing the nutrient concentration experienced by the inner cells.

All that can be definitely concluded from these results is that ammonium additions typically enhanced periphyton growth in the streams studied. These increases in algal biomass were small, however, approximately 50% above controls. In experiments indicating nitrogen limitation, nitrogen additions in either form increased algal biomass on the average 100% above controls.

Nutrient levels appeared to be very dynamic in the agricultural systems studied. In the two experiments which demonstrated nitrogen limitation, ambient nitrogen levels were greatly reduced. Both experiments were characterized by extended low flows, warm water temperatures and infrequent storm events. Under these conditions, algal mats typically cover most of the stream bed. These large algal mats could be an important factor in reducing ambient nutrient levels. Photosynthetic activities associated with benthic algal communities have been shown to affect the water chemistry of these streams. Kortge (1984), in a study on Big creek, Iowa, found that on average, plant photosynthesis produced $10 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, which is equivalent to 3.1 g C. A N:C ratio of 5.7 by weight would imply the capacity to remove

$550 \text{ mg m}^{-2} \text{ d}^{-1}$ of nitrogen from the water. With an average depth of 20 cm in this stream, the plants could theoretically remove 2.75 mg l^{-1} nitrogen from the water column each day.

The development of conditions leading to nutrient limitation appear to be frequently disrupted by storm events. Five of the fourteen experiments were deleted because of spates which occurred near the end of the experiment. Scouring associated with heavy rains was observed to remove algal mats from stream bottoms and effectively scour algae from substrata in these experiments. The role of storm events in disrupting periphyton community development has been discussed by other researchers (Fisher et al., 1982; Triska et al., 1983). An example of the scouring capability of storms can be found in data from deleted experiments. In one instance, discharge increased 500-fold overnight leaving only residual algal populations where algal mats had been the day before. Rains also tend to increase nitrate levels in agricultural streams. In the experiment mentioned above, a 50-fold increase in nitrate levels accompanied the increase in discharge. Smaller rain events may have little impact on the conditions leading to nitrogen limitation.

Although nitrogen levels were greatly reduced in some experiments, the concentrations would not be considered limiting in most aquatic ecosystems. As discussed above, a vertical gradient in nutrient concentrations may exist within periphyton communities (Hoagland et al., 1982). Saturation levels for nutrient uptake may also be higher in eutrophic than mesotrophic or oligotrophic systems.

This study demonstrated that water temperature is probably a more important factor in controlling periphyton growth rates in agricultural streams than nutrients. Kilkus et al. (1975) found that water temperature could also affect levels of suspended algae in agricultural streams. It is not surprizing that in an aquatic system where nutrients are typically at saturation levels, temperature and possibly light are important in governing biomass production.

Water temperature may have also affected algal response to ammonium additions. Water temper-

ature declined sharply after experiment 4 and remained near freezing during experiments 5 and 6. At such cold temperatures, the growth rate of algae is greatly reduced, and subsequently, the energy requirements are also reduced. Under these conditions, algal growth rates would likely be limited by temperature dependent biochemical reactions.

Seasonal changes in the species composition of algal communities could be another important factor in understanding the observed differences in algal response between experiments. Vollenweider (1971), for example, suggested that ammonium may not be a preferred nitrogen source for all algal species.

The different streams evaluated in this study were located in close proximity and conditions were generally similar between experiments. Likewise, periphyton response to nutrient additions were similar between streams. The exception would be Big Creek where nitrate levels were seldom reduced to the degree characterized by the other streams. The sampling areas were located in the headwaters of Big Creek where a significant proportion of flow can be attributed to tile drainage from adjacent agricultural fields. This water is typically high in nitrates (McDonald & Splinter, 1982; Iowa's Dept. Water Air and Waste Management, 1984).

Acknowledgements

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