

## Experimental evidence for nitrogen limitation in a northern Ozark stream

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**Abstract.** Nutrient enrichment experiments were conducted during low flow periods in 1985 and 1986 in a northern Ozark stream characterized by low nitrate-N concentrations and molar ratios of TN:TP less than 20:1. Enrichment with nitrate to a concentration 3–6 times greater than ambient, either alone or in combination with phosphate addition, stimulated periphytic chlorophyll *a* by 4–6× and AFDM by 2–5× over unenriched controls in two 30-d experiments. An intermediate increase in chlorophyll *a* in response to phosphate addition was observed in 1985 when TN:TP averaged 19:1. Differences in periphyton accrual among treatments were influenced by snail grazing (*Goniobasis*) during 1986 when periphyton biomass in N-enriched treatments continued to increase over the 30-d enrichment period, but remained at low levels in control and P-enriched treatments. Low nitrate-N concentrations and TN:TP ≤ 20:1 were also characteristic of 16 sites on 10 streams in the northern Ozark Plateau during low flow periods in 1985 and 1986, suggesting that nitrogen limitation may be common in the region.

**Key words:** periphyton, nitrogen limitation, N:P ratio, grazing, *Goniobasis*, Ozarks.

The most convincing evidence for nutrient limitation in streams comes from experimental studies in which periphyton growth has been stimulated by nutrient additions. Three techniques have generally been used to test for nutrient limitation in streams: whole-stream nutrient enrichment (Gregory 1980, Elwood et al. 1981), flow-through systems of parallel troughs dosed with various nutrient regimes (Stockner and Shortreed 1978, Manuel and Minshall 1981, Peterson et al. 1983, Triska et al. 1983, Tate 1985, Grimm and Fisher 1986a), and nutrient-diffusing substrata (Pringle and Bowers 1984, Fairchild et al. 1985, Tate 1985; Grimm and Fisher 1986a, Hill and Knight 1988, Bushong and Bachmann 1989).

Most studies demonstrating nutrient limitation in streams have identified either nitrogen (N) or phosphorus (P) as the limiting element. If nutrient concentrations are low enough to be limiting, then the relative abundance of N and P likely determines which of these elements limits algal growth. Based on cellular proportions of nitrogen and phosphorus in marine phytoplankton, Redfield (1958) proposed an atomic ratio of 16:1 as a transition point from N to P limitation. Thus, when the N:P ratio in the surrounding environment exceeds 16:1, P

potentially limits algal growth; when the N:P ratio is less than 16:1, algae are potentially N-limited. In bioassays of river periphyton communities, Schanz and Juon (1983) found N limiting at N:P < 10:1, P limiting at N:P > 20:1, and at ratios between 10–20:1 that either element might be limiting. Experimental enrichment studies have generally confirmed these estimates, with P additions stimulating periphyton growth at N:P > 20:1 (Stockner and Shortreed 1978, Elwood et al. 1981, Peterson et al. 1983, Pringle 1987) and response to N enrichment reported at N:P < 10:1 (Gregory 1980, Grimm and Fisher 1986a, Hill and Knight 1988).

Although P has more often been proposed as the limiting element in freshwater ecosystems (Hecky and Kilham 1988), N limitation was documented by Grimm and Fisher (1986a) in Sycamore Creek, Arizona; and based on a survey of N:P ratios in 157 Arizona streams, Grimm and Fisher (1986b) suggested that N limitation was likely the rule, rather than the exception, in streams of the desert Southwest. Nitrogen has also been identified as the potential limiting element in streams in northern California and the Pacific Northwest, where both NO<sub>3</sub>-N concentrations and N:P ratios are typically low (Thut and Haydu 1971, Gregory 1980, Triska et al. 1983, Hill and Knight 1988). Jones et al. (1984) hypothesized that based on low N:P ratios, algal biomass in about a third of the streams in the

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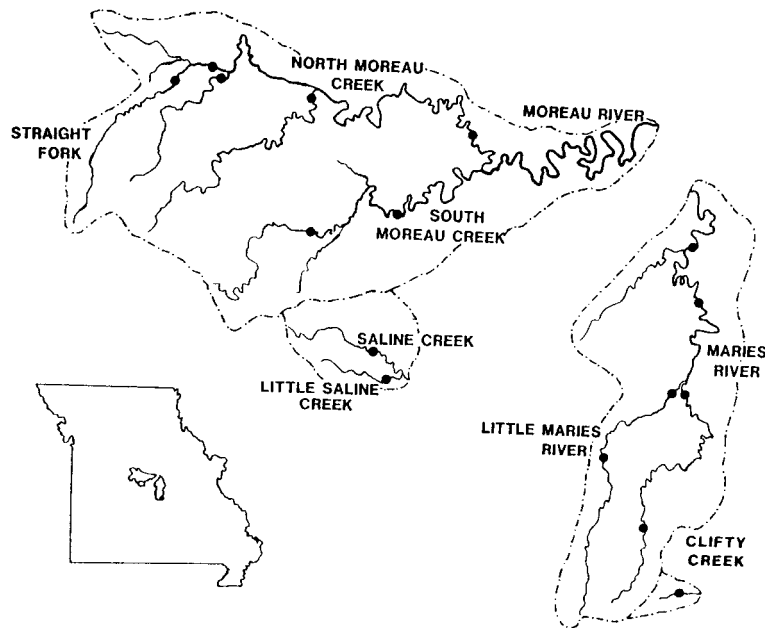


FIG. 1. Sixteen stream sites in the northern Ozark Plateau sampled during 1985-1986.

Missouri Ozark Plateau might be N-limited. Our objective in this study was to test experimentally whether N or P limits algal growth in an Ozark stream by measuring periphyton response to manipulated N and P concentrations in a flow-through system of parallel troughs. We also surveyed streams in the northern Ozark Plateau to determine, based on N:P ratios, the extent of potential nitrogen limitation of periphyton productivity in the region.

### Study Sites

#### *Saline Creek*

Nutrient enrichment experiments were conducted in Saline Creek, a 2nd-order stream in the northern Ozark Plateau of central Missouri. In 1985-1986, land use in the 56 km<sup>2</sup> watershed was 61% forest, 30% pasture, and 5% row crop agriculture. Mean annual discharge is 0.40 m<sup>3</sup>/s (based on bimonthly measurements from March-November in 1985-1986), with peak flows generally occurring during spring and late fall and low flow periods extending from summer through early fall. Along most of its length, Saline Creek is 5-10 m wide and depths rarely exceed 1 m in pools or 10-20 cm in riffles. Substrate is primarily gravel and cobble, with

patches of sand, bedrock, and boulder. Saline Creek is characterized by extensive gravel flats which create areas of open canopy along much of the stream. The water is clear (total suspended solids of less than 1.0 mg/L except during periods of high water) and well-buffered (the range of alkalinity is 138-225 mg CaCO<sub>3</sub>/L).

#### *Northern Ozark streams*

During 1985-1986, water samples were collected at 16 sites on 10 streams (including Saline Creek) in the northern Ozark Plateau (Fig. 1). All sites are within 100 km of Saline Creek. Physical and chemical conditions in the streams sampled are generally similar to those found in Saline Creek. Stream size ranges from 2nd- to 4th-order and watershed area ranges from 20.3 to 239.5 km<sup>2</sup>. Land use was 11-66% forest, 26-62% pasture, and 5-43% row crop agriculture. Detailed site descriptions are given in Lohman (1988).

### Methods

A flow-through system of parallel wooden troughs was used to measure the response of periphyton to additions of N and P during two 30-d periods in 1985 and 1986. The apparatus

TABLE 1. Mean total phosphorus (TP), total nitrogen (TN), and nitrate ( $\text{NO}_3\text{-N}$ ) concentrations in troughs during 30-d enrichment experiments in 1985 and 1986.

Treatment	Year	TP ( $\mu\text{g/L}$ )	TN ( $\text{mg/L}$ )	$\text{NO}_3\text{-N}$ ( $\text{mg/L}$ )	TN: TP (molar)
C	1985	20	0.17	0.09	19:1
	1986	21	0.11	0.05	12:1
+P	1985	41	0.17	0.09	9:1
	1986	38	0.11	0.05	6:1
+N	1985	20	0.35	0.27	39:1
	1986	21	0.39	0.34	41:1
+NP	1985	38	0.37	0.29	22:1
	1986	40	0.35	0.29	19:1

consisted of four troughs (each 3.5 m long, 28 cm wide, and 30 cm deep) placed on the stream bottom. Nutrient solutions in two 50-L and one 20-L carboy were contained in a box that rested on the upstream end of the troughs. Solutions were dripped from the carboys into the troughs with adjustable stopcocks, and baffles were used to insure mixing. Flow of Saline Creek water through the troughs was regulated to maintain depths between 12 and 20 cm and current velocities between 10 and 15 cm/s.

The first experiment was run from 27 August to 26 September 1985. Troughs were designated as control (C), nitrogen (N), phosphorus (P), and nitrogen plus phosphorus (N + P) treatments. A sodium nitrate stock solution (21.0 g  $\text{NO}_3\text{-N/L}$ ) was used to enrich the N treatment, and phosphoric acid (3.6 g  $\text{PO}_4\text{-P/L}$ ) was used as the source of phosphate in the P treatment. Concentrations in the N + P stock solution were 21.0 g  $\text{NO}_3\text{-N/L}$  and 1.8 g  $\text{PO}_4\text{-P/L}$ . Drip rates in enriched treatments were adjusted with changes in flow and ambient concentration to maintain TN nominally at triple and TP at double ambient concentrations in Saline Creek (Table 1).

Bare rocks from nearby gravel bars were placed in the troughs on day 0 of the enrichment. Two sets of eight rocks each were removed from each trough after 11, 21, and 30 d for analysis of chlorophyll *a* and ash-free dry mass (AFDM). Periphyton was scraped from a 5.3-cm<sup>2</sup> area on each rock, rinsed into a scintillation vial and later collected on filters for either chlorophyll *a* or AFDM analysis. Chloro-

phyll *a* was measured fluorometrically and corrected for phaeopigments by acidification, after extraction in a 50:50 mixture of dimethyl sulfoxide (DMSO) and 90% acetone in the dark for 24 h (Shoaf and Lium 1976, Knowlton 1984). AFDM was calculated as weight loss following ignition at 550°C for 1 h after samples had been dried to constant weight on Whatman GF/C filters at 105°C (APHA 1984). Periphyton was scraped from three additional rocks on each sampling date and examined microscopically to describe any changes in algal community composition. Biovolume was estimated for each of the 21 most common genera by measuring cell dimensions of 30 cells, calculating a mean cell volume, and multiplying this value by cell number.

Troughs were exposed to full sunlight for at least 6 h each day (roughly 9:00 to 15:00) and were only partially shaded during early morning and late afternoon. Water temperatures at midday declined during the 30-d enrichment period from 25°C to 14°C. Within troughs, the range of pH was 7.2–7.7 over the 30-d period, but identical measurements among troughs on a given sampling date indicated that acidic enrichment solutions had no effect on pH. Mean flow through each of the four troughs ranged from 4.8 to 5.5 L/s over the 30-d period.

Nutrient concentrations were monitored in each trough every 2–3 d. TP was analyzed using the ascorbic acid method after persulfate oxidation (Prepas and Rigler 1982).  $\text{NO}_3 + \text{NO}_2\text{-N}$  was determined by cadmium reduction and TN by cadmium reduction after persulfate oxidation (D'Elia et al. 1977).  $\text{NO}_2\text{-N}$  and  $\text{NH}_4\text{-H}$  were below levels of detection in initial samples (0.01 mg/L) and were not analyzed in subsequent samples. Values reported here for  $\text{NO}_3\text{-N}$  are those obtained for  $\text{NO}_3 + \text{NO}_2\text{-N}$ , on the assumption that  $\text{NO}_2\text{-N}$  concentrations throughout the experiment were negligible.

Enrichment was repeated 16 July–15 August 1986 with only minor variation. Length of the troughs was increased to 4.8 m, permitting the number of samples collected for chlorophyll *a* and AFDM to be increased from eight to nine on each sampling date. Nitric acid was used in place of sodium nitrate as a source of N enrichment because it was easier to mix and keep in solution and because use of phosphoric acid the previous year had indicated that acidic stock solutions would not alter pH. Midday water

temperatures were warmer and more constant over the 30-d period than in 1985, ranging from 27°C on the first day of enrichment to 26°C on the last day. Average flow through each trough over the 30-d period was similar among treatments (4.8–5.3 L/s).

The snail *Goniobasis* sp. was an unanticipated addition to the experimental design in 1986. A few were present in troughs in 1985, but never in numbers that we considered large enough to affect periphyton biomass. In 1986, however, snails were prevalent in all treatments within a few days after the experiment began and densities were high enough to potentially lower periphyton biomass. On day 23, after attempts to exclude *Goniobasis* were unsuccessful, we estimated snail densities by removing all rocks from the last 50-cm length of each trough and counting the snails. The same method was used to census snails on day 30 when the experiment was concluded.

We present the results of these two experiments by comparing mean levels of chlorophyll *a* and AFDM on each sampling date. Although replicate subsamples were taken from each treatment on each sampling date, only one trough was used for each treatment in both 1985 and 1986, and thus our experiments are not replicated. Heeding Hurlbert's (1984) admonitions, no inferential statistics comparing treatment effects are presented.

Water samples were collected bimonthly from 16 sites on 10 streams in the northern Ozark Plateau from March–November in 1985 and 1986. Total nitrogen, total phosphorus, and nitrate-N were analyzed by the methods previously described. Mean nutrient concentrations, excluding samples affected by recent storm events, were calculated for low flow periods for each site in both years (July–October 1985, June–September 1986).

## Results

### *Saline Creek enrichment experiments*

Chlorophyll *a* was similar among treatments after 11 d of enrichment in 1985, but higher in enriched treatments than in the control after 21 d (Fig. 2A). After 30 d, chlorophyll *a* was highest in N and N + P (79.8, 77.3 mg/m<sup>2</sup>), intermediate in P (46.0 mg/m<sup>2</sup>), and lowest in the control (20.0 mg/m<sup>2</sup>). Nitrogen enrichment also stim-

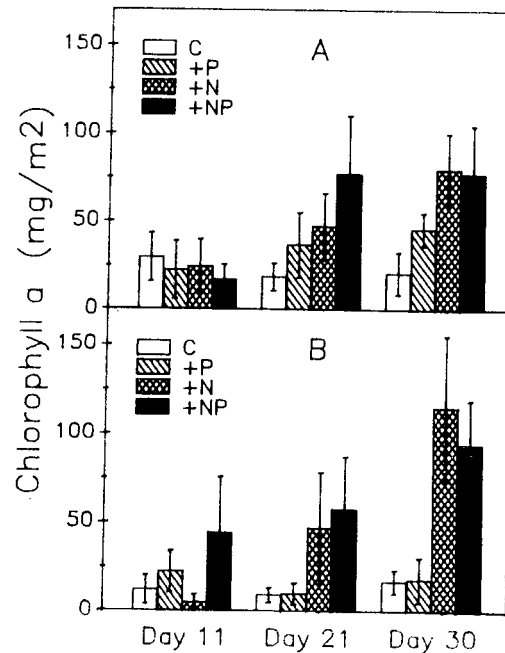


FIG. 2. Benthic chlorophyll *a* (mean  $\pm$  1 SD) after 11, 21, and 30 d in control (C), phosphorus (+P), nitrogen (+N), and nitrogen plus phosphorus (+NP) treatments. (A) 27 August–26 September 1985,  $n = 8$  subsamples per treatment, (B) 16 July–15 August 1986,  $n = 9$  subsamples per treatment.

ulated chlorophyll *a* standing crops when the experiment was repeated in 1986 (Fig. 2B). Chlorophyll *a* was noticeably higher in nitrogen-amended treatments after 21 d, and was roughly six times higher in N and N + P treatments (115.0, 94.0 mg/m<sup>2</sup>), than in P and control troughs (17.9, 16.9 mg/m<sup>2</sup>) after 30 d. Similar differences among treatments were observed in both years when AFDM was used as an estimate of periphyton standing crop (Fig. 3).

Pigment quality differed more between years than among treatments. Phaeophytin, as a percent of total pigment concentration, was 12.3–22.0% among all treatments over the 30-d enrichment period in 1985 in contrast to 22.4–40.6% in 1986 (Table 2). Mean chlorophyll *a*:AFDM was 8.3 mg/g in 1985 (average of all treatments on three sampling dates), but differences among treatments were not discernible. Chlorophyll *a*:AFDM was consistently lower in C and P (mean = 4.3 mg/g) than in N and N + P (mean = 8.4 mg/g) treatments in 1986.

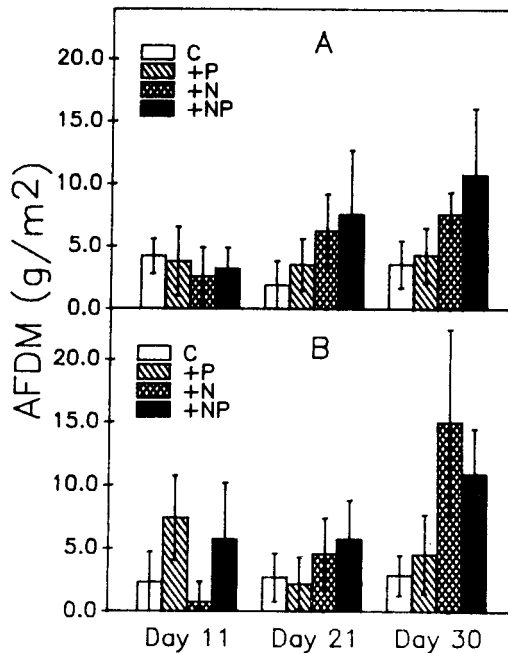


FIG. 3. Benthic AFDM (mean  $\pm$  1 SD) after 11, 21, and 30 d in control (C), phosphorus (+P), nitrogen (+N), and nitrogen plus phosphorus (+NP) treatments. (A) 27 August-26 September 1985,  $n = 8$  subsamples per treatment, (B) 16 July-15 August 1986,  $n = 9$  subsamples per treatment.

Forty algal genera were identified in 1985, but community composition did not differ clearly among treatments. Diatoms dominated all samples in 1985, making up 85-97% of algal biovolume. *Achnanthes* was the most common diatom in all treatments after 30 d, making up 29-58% of the diatom community. Of the 40 taxa identified in 1985, only 22 were seen during the 1986 experiment. None of the missing taxa (10 diatoms, 6 greens, and 2 blue-greens) had been abundant in 1985. Early colonization in the second experiment was dominated by diatoms (*Navicula*, *Cocconeis*) and by the chrysophyte *Bumilleria*. Diatom communities on day 30 were dominated by *Gomphonema*, *Cocconeis*, and *Achnanthes*.

*Goniobasis* appeared within a few days of the beginning of the experiment in 1986 and was prevalent in all treatments by the first sampling date (day 11). Snail densities on day 23 averaged 1054/m<sup>2</sup> and declined over the last week of the experiment to an average of 666/m<sup>2</sup> among treatments on day 30 (Table 3).

TABLE 2. Phaeophytin (mean  $\pm$  1 SD), as a percent of total pigment concentration, in control (C), phosphorus (+P), nitrogen (+N), and nitrogen plus phosphorus (+NP) treatments after 11, 21, and 30 d of enrichment in 1985 and 1986 ( $n = 8$  in 1985,  $n = 9$  in 1986).

Treatment	Day 11	Day 21	Day 30
1985			
C	18.3 $\pm$ 4.2	12.3 $\pm$ 8.0	13.5 $\pm$ 8.1
+P	22.0 $\pm$ 7.6	20.0 $\pm$ 6.4	16.8 $\pm$ 3.1
+N	21.7 $\pm$ 6.2	14.8 $\pm$ 8.0	21.0 $\pm$ 4.2
+NP	18.0 $\pm$ 6.1	19.4 $\pm$ 9.5	15.6 $\pm$ 4.5
1986			
C	40.6 $\pm$ 6.7	24.8 $\pm$ 11.5	32.5 $\pm$ 4.5
+P	37.4 $\pm$ 4.6	27.7 $\pm$ 7.1	22.4 $\pm$ 7.7
+N	29.0 $\pm$ 11.5	33.8 $\pm$ 8.7	38.4 $\pm$ 6.4
+NP	30.9 $\pm$ 6.3	34.1 $\pm$ 6.5	36.1 $\pm$ 5.6

#### Nutrient chemistry in northern Ozark streams

Streams surveyed in the northern Ozark Plateau were generally characterized by low TN and NO<sub>3</sub> + NO<sub>2</sub>-N concentrations during low flow periods in both 1985 and 1986 (Tables 4, 5). NO<sub>3</sub> + NO<sub>2</sub>-N was particularly low during summer 1986 when concentrations  $\leq$  0.02 mg/L were recorded at all but two sites. At 8 of 16 sites in 1985 and at 10 of 26 sites in 1986, TN:TP ratios were  $\leq$  20:1 throughout low flow periods.

#### Discussion

Nitrate additions stimulated periphyton growth, measured as either chlorophyll *a* or AFDM, in both experiments. Increasing nitrate

TABLE 3. Density of snails (*Goniobasis* sp.) in control (C), phosphorus (+P), nitrogen (+N), and nitrogen plus phosphorus (+NP) treatments after 23 and 30 d of enrichment in 1986.

Treatment	Number/m <sup>2</sup>	
	Day 23	Day 30
C	1135	661
+P	885	562
+N	1048	836
+NP	1147	603

TABLE 4. Means and ranges of total nitrogen (TN), nitrate + nitrite ( $\text{NO}_3 + \text{NO}_2\text{-N}$ ), total phosphorus (TP), and TN:TP ratios at 16 sites on 10 streams in the northern Ozark Plateau during summer low flow in 1985 (July–October).

Site	n	TN (mg/L)		$\text{NO}_3 + \text{NO}_2\text{-N}$ (mg/L)		TP ( $\mu\text{g/L}$ )		TN:TP (molar)		% Times sampled TN:TP $\leq 20:1$
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Saline Creek drainage										
Saline Creek	8	0.23	0.08–0.38	0.07	0.02–0.12	21	13–38	25	12–31	12.5
L. Saline Cr.	9	0.52	0.34–0.70	0.43	0.28–0.60	26	22–38	43	34–56	0.0
Moreau River drainage										
Straight Fork	8	0.27	0.18–0.42	0.04	0.02–0.08	56	35–76	10	9–13	100.0
Smiths Creek	8	0.26	0.14–0.46	0.04	0.01–0.09	50	32–74	11	8–15	100.0
Burris Fork	8	0.24	0.16–0.34	0.02	0.01–0.05	58	41–78	9	5–12	100.0
N. Moreau Creek										
Site 1	8	0.30	0.18–0.44	0.03	0.01–0.11	77	60–110	9	5–12	100.0
Site 2	7	0.41	0.26–0.48	0.04	0.02–0.05	60	43–75	15	11–20	100.0
S. Moreau Creek										
Site 1	8	0.21	0.16–0.30	0.04	0.01–0.16	47	34–56	10	9–16	100.0
Site 2	8	0.27	0.22–0.39	0.02	0.01–0.05	69	56–102	9	8–10	100.0
Maries River drainage										
Maries River										
Site 1	8	0.28	0.18–0.43	0.16	0.12–0.30	15	11–22	43	20–73	12.5
Site 2	8	0.21	0.08–0.28	0.09	0.02–0.20	15	10–20	31	15–52	12.5
Site 3	8	0.18	0.09–0.30	0.04	0.01–0.16	16	9–16	27	8–39	12.5
Site 4	8	0.19	0.06–0.28	0.02	0.01–0.04	37	31–51	12	4–17	100.0
L. Maries River										
Site 1	8	0.13	0.03–0.20	0.04	0.02–0.08	9	6–12	33	7–59	12.5
Site 2	8	0.18	0.03–0.33	0.09	0.01–0.23	14	8–22	29	8–67	12.5
Clifty Creek	8	0.18	0.04–0.30	0.11	0.04–0.16	5	3–7	83	13–163	12.5

concentrations by 3–6 times over background levels, with or without additional phosphate, increased chlorophyll *a* by 4–6 $\times$  and AFDM by 2–5 $\times$  the amounts measured in unenriched controls. From these responses, we conclude that nitrogen was the primary limiting nutrient in Saline Creek during summer low flow periods.

Our results are consistent with studies that have shown nitrogen limitation in streams with low N:P ratios. Grimm and Fisher (1986a) demonstrated that nitrogen limited periphyton productivity in an Arizona stream characterized by N:P ratios of 1.6–2.6 (based on  $\text{NO}_3\text{-N}:\text{SRP}$ ). In a heavily-shaded stream in the Pacific Northwest, where ratios of inorganic N to inorganic P were 0.2–2.0, Gregory (1980) reported increased chlorophyll *a* accrual in response to nitrate addition when accompanied by artificial lighting. Hill and Knight (1988) found en-

hanced algal accrual on nitrate-diffusing substrates in an unshaded northern California stream where  $\text{N:P} < 2$ . Increased biomass accumulation in response to nitrate and phosphate enrichment, as well as high rates of nitrate uptake by periphyton, strongly supported the conclusions of Triska et al. (1983) and Sebetich et al. (1984) that periphyton is N-limited in Little Lost Man Creek, California, where  $\text{NO}_3\text{-N}:\text{PO}_4\text{-P} < 1$ .

Nitrogen stimulated periphyton growth in Saline Creek at N:P ratios an order of magnitude higher than in any previous stream study reporting N limitation. Nutrient conditions in Saline Creek were likely near the upper bound of N:P ratios where N limitation would be expected. The intermediate increase in chlorophyll *a* in response to P addition in 1985, when TN:TP averaged 19:1, suggests that P, rather

TABLE 5. Means and ranges of total nitrogen (TN), nitrate + nitrite (NO<sub>3</sub> + NO<sub>2</sub>-N), total phosphorus (TP), and TN:TP ratios at 16 sites on 10 streams in the northern Ozark Plateau during summer low flow in 1986 (June–September).

Site	n	TN (mg/L)		NO <sub>3</sub> + NO <sub>2</sub> -N (mg/L)		TP (μg/L)		TN:TP (molar)		% Times sampled TN:TP ≤ 20:1
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Saline Creek drainage										
Saline Creek	7	0.10	0.06–0.16	0.05	0.01–0.08	17	12–22	14	9–26	85.7
L. Saline Cr.	7	0.36	0.24–0.58	0.29	0.18–0.47	27	24–35	29	21–37	0.0
Moreau River drainage										
Straight Fork	8	0.28	0.22–0.34	0.04	<0.01–0.08	76	50–184	10	3–13	100.0
Smiths Creek	8	0.28	0.20–0.34	0.03	<0.01–0.06	50	35–66	13	9–16	100.0
Burriss Fork	8	0.58	0.36–1.16	0.13	<0.01–0.44	94	62–166	13	9–19	100.0
N. Moreau Creek										
Site 1	8	0.38	0.21–0.64	0.06	<0.01–0.24	91	62–170	9	8–11	100.0
Site 2	5	0.57	0.32–0.93	0.11	0.02–0.24	112	64–189	12	8–17	100.0
S. Moreau Creek										
Site 1	6	0.31	0.27–0.35	0.02	<0.01–0.10	56	38–68	12	10–18	100.0
Site 2	4	0.36	0.32–0.44	0.01	<0.01–0.03	100	88–126	8	8–8	100.0
Maries River drainage										
Maries River										
Site 1	8	0.18	0.10–0.28	0.05	0.01–0.13	14	6–24	31	17–52	25.0
Site 2	5	0.11	0.04–0.16	0.03	0.01–0.06	22	16–28	11	4–14	100.0
Site 3	5	0.14	0.11–0.16	0.03	0.02–0.06	23	18–28	13	12–17	100.0
Site 4	5	0.18	0.14–0.23	0.01	<0.01–0.02	42	34–52	10	7–12	100.0
L. Maries River										
Site 1	7	0.14	0.08–0.18	0.06	<0.01–0.14	11	8–16	29	19–44	42.8
Site 2	7	0.18	0.06–0.27	0.08	0.02–0.19	18	10–24	22	7–28	14.2
Clifty Creek	9	0.25	0.10–0.53	0.19	0.08–0.40	8	3–16	81	30–147	12.5

than N, would limit periphyton growth at higher N:P ratios.

The clear differences in accrued biomass that distinguished N and N + P from C and P-enriched troughs in 1986 were probably accentuated by the intense grazing pressure in the second experiment. Numerous studies have demonstrated the ability of grazers, and particularly snails, to limit algal standing crops in streams (Elwood and Nelson 1972, Gregory 1980, Jacoby 1985, Hawkins and Furnish 1987, Feminella et al. 1989, McCormick and Stevenson 1989). Based on the number of snails counted in the troughs on days 23 and 30, *Goniobasis* density averaged 860/m<sup>2</sup> during the latter part of the experiment. Malone and Nelson (1969) measured grazing rates of *Goniobasis clavaeformis* in laboratory streams of 0.018 mg AFDM/snail/h or 0.432 mg AFDM/snail/day, which Elwood

and Nelson (1972) used to estimate grazing by *G. clavaeformis* in Walker Branch, Tennessee. Assuming a similar rate in Saline Creek, grazing would account for a loss of 372 mg AFDM/m<sup>2</sup>/day or roughly 8–17% of periphyton biomass in C and P troughs measured on days 21 and 30. If net primary production rates are assumed to be 5–20% of periphyton standing crop (Elwood and Nelson 1972), then grazing and production rates would have been comparable, and periphyton standing crop could have been limited by grazers. In contrast, biomass increased throughout the experiment in N and N + P treatments, outrunning grazing by *Goniobasis*. Using the grazing rate estimate of 372 mg AFDM/m<sup>2</sup>/d, grazing losses would have been 5–8% of periphyton standing crop on day 21 and 2–3% on day 30 in N and N + P treatments.

Grazing intensity may explain the smaller

number of algal taxa seen during the second experiment in 1986. High snail density can reduce species diversity in benthic algal communities (Lowe and Hunter 1988, Marks and Lowe 1989, McCormick and Stevenson 1989) and may have been responsible for eliminating rare taxa from samples collected in 1986. Differences in the time of year that the experiments were conducted might also account for taxonomic differences between years. Water temperature remained relatively warm and constant in 1986, whereas a steady decline in temperature occurred in 1985, perhaps signaling a shift from a warm-summer algal community to a cooler-fall one.

Greater accumulation of detritus associated with heavy grazing pressure may explain why the proportion of phaeophytin to total pigment was roughly twice as high in all treatments in 1986 as in 1985. The lower chlorophyll *a*:AFDM ratios in control and P-enriched troughs as compared to N and N + P-enriched treatments in 1986 may indicate N deficiency in algal cells where chlorophyll *a* synthesis is reduced (Healey and Hendzel 1979).

Ratios of TN:TP provide only a rough estimation of actual N:P supply ratios to stream periphyton. Indices that include particulate, as well as inorganic fractions of N and P, overestimate the biotic availability of both nutrients. In contrast, using ratios of only the inorganic fractions (e.g., DIN:SRP) likely underestimates the external supply of both N and P. In testing nine indices for accuracy in predicting nutrient limitation in eight Colorado lakes, Morris and Lewis (1988) found the ratio of DIN:TP to be a better predictor than either TN:TP or DIN:SRP and suggested that particulate P is more readily available to phytoplankton than is particulate N. A transitional point between N and P limitation based on TN:TP may therefore be higher than the Redfield (1958) ratio of 16:1.

N limitation of periphyton growth is likely common in northern Ozark streams in light of the stimulatory effects of N in Saline Creek and the widespread occurrence of TN:TP < 20:1 in streams of the region during low flow periods. Low NO<sub>3</sub> + NO<sub>2</sub>-N concentrations during these periods support this conclusion. The limited availability of N in northern Ozark streams could also explain the dominance of blue-green algae, which make up 60.8–79.3% of periphyton

assemblages in these streams during late summer and early fall (Baysinger-Daniel 1989).

Conditions favoring N limitation seem most likely to develop in streams subject to long periods of low discharge, like those that can occur in northern Ozark streams and in Arizona streams (Grimm and Fisher 1986a, 1986b). In nutrient-rich agricultural streams in Iowa, where there was no indication of nutrient limitation in 7 of 9 experiments using nutrient-diffusing substrata, there was evidence of nitrogen limitation in two experiments conducted during extended low flows (Bushong and Bachmann 1989). Denitrification can account for significant losses of N during low flow (Hill 1981, 1983) and may explain more rapid declines in ambient concentrations of nitrate than phosphorus. In addition, abiotic factors can control phosphorus concentrations, and sediments may provide a phosphorus source during low flow (Meyer 1979, Grimm et al. 1981), further contributing to differential declines in ambient concentrations of N and P, and increasing the potential for N limitation.

In a nationwide survey, Omernik (1977) reported trends of higher phosphorus and lower nitrogen concentrations in streams of the central (including the Ozark Plateau) and western United States in relation to streams in the eastern part of the country. These trends may help explain why previous stream studies demonstrating N limitation have generally been geographically restricted to the western states. In several of these studies (Grimm and Fisher 1986a, 1986b, Marcus 1980, Thut and Haydu 1971), high stream P concentrations have been attributed to watersheds of volcanic origin, which are often rich in phosphorus (Dillon and Kirchner 1975). Streams in the northern Ozark Plateau drain watersheds with limestone-dolomite parent material (Collier 1953) and streams draining watersheds of sedimentary geology also tend to be phosphorus-rich (Dillon and Kirchner 1975). Thus, watershed geology may be a major factor responsible for regional differences in stream nutrient limitation.

Grimm and Fisher (1986a), in assessing the potential for nitrogen limitation in Arizona streams, suggested a NO<sub>3</sub>-N concentration of 0.055 mg/L or below as a limiting concentration. Our results suggest that nitrogen limitation can occur in Saline Creek at NO<sub>3</sub>-N levels at least as high as 0.10 mg/L. Low NO<sub>3</sub>-N con-



centrations and low TN:TP ratios during summer and fall in other streams in the northern Ozark Plateau, as well as in those of the southern Ozark Plateau (Jones et al. 1984), indicate that nitrogen limitation is probably a common phenomenon in the area. Although phosphorus has long been assumed to be the primary limiting nutrient in aquatic ecosystems, the growing number of studies that have reported algal responses to nitrogen enrichment suggest that nitrogen availability is an important factor in regulating stream primary productivity in many regions.

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