Nutrient limitation of algal biomass accrual in streams: seasonal patterns and a comparison of methods

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Abstract. In-situ, nutrient amendment experiments (nutrient-diffusing substrata, NDS) were conducted in 12 New Zealand gravel-bed streams to investigate seasonality of biomass accrual and nutrient limitation of benthic algal communities. Benthic algal biomass accrual rates exhibited significant ($p = 0.019$, repeated measures ANOVA) seasonal differences; rates were greatest in summer and least in winter. The degree of nutrient limitation also differed ($p = 0.003$) seasonally; periphyton community biomass was most responsive to nutrient amendments in summer and least responsive in winter. Temperature may be the underlying cause of these patterns. The ratios of dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) in streamwater and of streambed periphyton communities were of limited use for predicting which nutrient limited NDS bioassays; cellular nutrient content was weakly predictive. This study demonstrates the need to consider temporal changes (i.e., seasonality) when assessing the influence of nutrients on stream ecosystems, and indicates that the use of nutrient ratios to ascertain which nutrient may limit benthic algal biomass should be validated with field experiments.

Key words: benthic algae, periphyton, biomass, nutrients, seasonality, nutrient limitation, nutrient ratios, cellular nutrients.

Flood disturbance and nutrient resource supply are fundamental determinants of broadscale patterns in lotic primary production and periphyton community composition (review by Biggs 1996, Biggs et al. 1998b). Biomass can be severely reduced and taxonomic structure shifted toward low-profile, tightly adhering taxa by floods (e.g., Stevenson 1990, Uehlinger 1991, Biggs 1995, Biggs et al. 1998a, 1998b). In contrast, high nutrient supply tends to stimulate growth rates of certain populations, leading to high biomass communities of erect, tall-growing taxa in unshaded streams during extended periods without disturbances (e.g., Lowe et al. 1986, Bothwell 1989, Lohman et al. 1991). Overall, flood disturbance and nutrient resource supply can explain between 66% and 86% of the annual variance in mean monthly biomass among gravel–cobble-bed streams covering wide geographic regions (Biggs 1988a, Biggs and Close 1989, Biggs 1995).

Many lotic systems display seasonal changes in benthic algal standing crop and community composition (e.g., Gumtow 1955, Biggs 1988b, Cox 1990a, 1990b) and primary productivity (e.g., Marker 1976, Hornick et al. 1981, Keithan and Lowe 1985, Antoine and Benson-Evans 1985). Several studies (Butcher 1946, Stockner and Shortreed 1976, Gale et al. 1979, Lowe and Gale 1980, Cox 1990b) have explicitly addressed year-round patterns in benthic algal biomass accrual. Investigations of periphyton seasonality have been sufficiently numerous and varied to allow generalized syntheses (see Cox 1990a, Biggs 1996), which have concluded that algal communities respond to seasonal variations in flow regime, nutrient supply, grazers, light, or temperature.

Analysis of the degree of nutrient limitation of periphyton growth over an annual cycle may indicate whether seasonal variation in biomass accrual is driven by altered nutrient supply. Nu-

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trient limitation of lotic periphyton community biomass is a well-studied subject (see Borchardt 1996). However, most such studies have been confined to relatively warm periods of time, typically midsummer. Only Winterbourn (1990), Stanley et al. (1990), Allen and Hershey (1996), and Biggs et al. (1998a) have conducted year-round, in-situ experiments (each in a single stream) investigating seasonal patterns of periphyton nutrient limitation. Additional efforts are required to ascertain how seasonal patterns of nutrient supply and nutrient limitation may influence the annual dynamics of periphyton communities in streams.

The way in which nutrient limitation is assessed may have an important bearing on our understanding of nutrient-driven periphyton dynamics. Many previous investigations have sought to relate the ratios of nutrients (typically N and P) in streamwater to the nutrient status of periphyton, in attempts to predict which nutrient is limiting algal community biomass. Streamwater N:P ratios have sometimes been useful indicators of which nutrient was limiting biomass (e.g., Schanz and Juon 1983, Grimm and Fisher 1986, Hill and Knight 1988, Peterson et al. 1993); however, such use of N:P ratios has not always been successful (e.g., Allen and Hershey 1996, Kutka and Richards 1997). Successes notwithstanding, inferring the nutrient status of algae by measuring nutrients in the water can be inaccurate for a variety of reasons (see Tilman et al. 1982, Schanz and Juon 1983, Biggs and Close 1989, Axler et al. 1994, Biggs 1995, Borchardt 1996), and more direct measures of algal nutrient status (i.e., analysis of cellular nutrient content, alkaline phosphatase activity, or nutrient-amendment experiments) are often advocated (e.g., Schanz and Juon 1983, Biggs and Close 1989, Biggs 1995, Borchardt 1996). Intercomparison of various methods of determining algal nutrient status in natural systems would allow evaluation of the degree of agreement amongst these methods.

The purpose of this study was 3-fold. First, we sought to identify seasonal patterns in benthic algal biomass accrual rates and associated nutrient limitation in 12 New Zealand headwater streams. Second, correlations with physical, chemical, and biological parameters were used to indicate possible causal mechanisms for the observed seasonal patterns. Last, we examined the relationships between streamwater N:P ra-

tios, cellular N:P ratios, cellular nutrient content, and results of nutrient-diffusing substrata (NDS) bioassays to assess the efficacy of using measurements of N:P ratios or cellular nutrient content to predict which nutrient (N or P) would limit biomass accrual on NDS bioassays.

Methods

Study sites

Twelve 3rd-order gravel-bed streams on the South Island of New Zealand were chosen for this study. These sites included streams with a range of bed stabilities and flood frequencies, and were sufficiently accessible for regular, year-round, sampling visits. Catchment geology was dominated by fine-grained metamorphic sandstone (greywacke or schist) and granites. The catchments of 6 streams were predominantly forested, whereas the remaining 6 catchments were mostly tall native tussock grasslands with low-intensity sheep grazing. All sampling sites received little direct shading during the day, but were partially shaded by vegetation and steep banks in the morning and late afternoon, particularly during winter. See Biggs et al. (1997, 1999) for additional site information.

Data collection

Hydrological data were collected as described by Biggs et al. (1997). Flood frequency at each site and time in flood during NDS incubations were calculated as the number of flood events or the number of days for which discharge exceeded 3 times the annual median for a given stream (Clausen and Biggs 1997). The maximum discharge of each stream during NDS incubations was expressed as a multiple of the median annual discharge of that stream.

Biological and chemical samples were collected during quarterly sampling visits in 1995 (summer: 9–17 January; autumn: 10–27 April; winter: 7–20 July; spring: 16–26 October). Sampling methods are detailed by Biggs et al. (1999). Briefly, water temperature was measured with a YSI DO meter (YSI Incorporated, Yellow Springs, Ohio, USA). Water samples were collected in acid-washed polyethylene bottles and transported to the laboratory on ice for analysis. Ten stones (size range ~40 to ~240 mm median diameter) from each stream were collected at

regular intervals along 2 transects (5 stones per transect) across the stream. The entire surface of each stone was scrubbed with a nylon-bristle brush. All material removed was rinsed into a single polyethylene bottle, placed in a darkened container, and transported to the laboratory on ice. Chemical, chlorophyll *a*, and ash-free dry mass (AFDM) analyses followed Biggs and Close (1989), Biggs (1995), and Biggs et al. (1999). Cellular nutrient concentrations were determined by analyzing periphyton subsamples for N and P as total Kjeldahl N and total P, correcting for N and P in the streamwater, and normalizing cellular nutrient content to AFDM to give cellular nutrient concentrations (%P_c and %N_c, Biggs and Close 1989). These cellular nutrient measures included all nutrients sorbed to inorganic particles and organic detritus, and nutrients present in all periphytic microorganisms (e.g., algae, bacteria, fungi, protozoa). The total area sampled for periphyton was determined by measuring the x, y, and z dimensions of each stone with calipers, and applying the regression equation of Biggs and Close (1989). Streamwater N:P was calculated as dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP, i.e., NO_2-N + NO_3-N + NH_4-N : PO_4-P), and both streamwater and cellular N:P ratios were expressed as atomic ratios. Throughout this study, NO_2-N + NO_3-N is reported as NO_3-N, because NO_2-N concentrations were negligible.

NDS bioassays (steel tray method of Biggs and Lowe 1994, Biggs et al. 1998a) were deployed in areas of similar velocity and flow conditions. Each bioassay consisted of 20 reservoirs filled with 2% agar (300 mL each), placed in 4 lines within a galvanized steel box (0.6 m × 0.4 m × 0.2 m, with an internal frame to hold the reservoirs). Agar in each of 5 replicate reservoirs was amended with each nutrient treatment, either 0.5 M $NaNO_3$ (N) or 0.05 M Na_2HPO_4 (P), a combination of 0.5 M $NaNO_3$ and 0.05 M Na_2HPO_4 (N + P), or no nutrient solution (C). Hardened, ashless filter papers were placed over the tops of the reservoirs to serve as a growth surface for periphyton. A flat lid with longitudinal partitions 2 cm high (to maintain parallel flow and prevent nutrient diffusion onto neighboring treatments) was secured to the top of the box. Holes, the diameter of the reservoir necks, allowed the lid to be seated so that the filter papers were flush with the surface of the lid and exposed to stream current. Substrata

were retrieved after a suitable incubation period (i.e., long enough to ensure adequate biomass development, but short enough to prevent autogenic sloughing; summer: 14–17 d, autumn: 18–21 d, winter: 22–27 d, spring: 17–18 d), and growth surfaces were removed and transported to the laboratory (in darkness, on ice) for chlorophyll analysis. Invertebrates present on the growth surfaces of the autumn, winter, and spring NDS bioassays were enumerated immediately following removal of bioassays from the streams. This protocol did not allow accurate counting of mobile invertebrate taxa (e.g., stoneflies and mayflies), but it was likely adequate for enumeration of more sessile taxa (in particular, caddisflies and chironomids), because removal from the stream did not appear to cause any loss of these organisms.

Data analysis

Nutrient limitation of benthic algal biomass accrual for each combination of stream and season was determined by applying 1-way ANOVA to data from the appropriate NDS bioassay (see Appendix 1 for results of individual experiments). To reduce heteroscedasticity, data were log(x + 1) transformed prior to analysis (Zar 1984). Tukey's HSD multiple comparison technique was used to calculate which treatments were significantly greater than others, thereby indicating which nutrient(s) were limiting. Nutrient limitation was inferred when significantly greater biomass (chlorophyll *a*) accrued on a nutrient-enriched treatment, relative to the control (no added nutrient) treatment; N + P co-limitation was considered to occur only for the bioassays in which the N + P treatment alone displayed significantly increased biomass. The degree of nutrient limitation of benthic algal biomass accrual was calculated for each NDS bioassay by calculating the ratio of mean biomass on nutrient-enriched substrata to mean biomass on control substrata (using whichever nutrient-enriched treatment displayed greatest biomass, almost always the N + P treatment). Thus, the degree of nutrient limitation reflected the extent to which overall nutrient availability (i.e., N + P, not N or P alone) limited algal biomass. Rates of benthic algal biomass accrual (under ambient nutrient conditions) for individual incubations were calculated by dividing the mean biomass on control (unenriched) substrata

TABLE 1. Hydrological conditions at the study sites during 1995. D_{84} floods = the number of flood events able to move the 84th percentile (by size) of the bed material at a given site (an integrated measure of bed stability and flood magnitude and frequency). Data from Biggs et al. (1997) and M. J. Duncan and B. J. F. Biggs (National Institute of Water and Atmospheric Research [NIWA], unpublished data).

Stream	NIWA hydro-metric station no.	Catchment area (km²)	Median discharge (m³/s)	Mean velocity (m/s)	Flood frequency (/y)	D_{84} floods (/y)
Bowyers	68818	23.2	0.61	0.39	27.0	4
Woolshed	68817	29.3	0.30	0.29	26.5	8
West Kowai	66413	19.6	0.38	0.65	4.3	3
North Kowai	66412	37.9	1.13	0.44	12.0	24
Kyeburn	74392	9.4	0.09	0.30	21.7	2
Timber	74393	13.9	0.26	0.39	14.5	15
Victoria	93236	8.5	0.45	0.47	29.3	16
Rough	93235	4.9	0.31	0.41	29.9	25
Granity	91423	7.6	0.18	0.36	46.2	16
Slaty	91424	18.1	1.00	0.43	43.5	13
Sams	91421	7.0	0.20	0.30	41.3	25
Camp	91422	6.9	0.62	0.40	40.0	27

by the incubation time (i.e., the net accrual rate of Stevenson 1996). Use of this calculation did not imply that the actual benthic algal biomass accrual rates were linear; it was used only to provide a simple, relative measure of biomass accrual rates.

Seasonal patterns in benthic algal biomass accrual rates, the degree of algal nutrient limitation, and invertebrate densities were assessed with univariate repeated measures ANOVA. All data were log(x + 1) transformed prior to analysis. The hypothesis that the proportion of streams displaying significant nutrient limitation (as indicated by NDS bioassays) did not differ among seasons was tested using a Chi-square contingency table.

Rates of biomass accrual and the degree of nutrient limitation were correlated with the maximum discharge during bioassay incubations, the number of days in flood during bioassay incubations, streamwater nutrient concentrations, conductivity, temperature, and the biomass and nutrient content of natural periphyton communities. Spearman's rank correlation method was used to minimize distributional assumptions. These correlation coefficients should be interpreted cautiously, because each site could contribute up to 4 data points (1 for each season) to the analysis. Even though they were collected at ~3-mo intervals, data from the same site may not be completely independent.

All analyses were done using Systat (version 5.2, SYSTAT Inc., Evanston, Illinois).

Results

Hydrological, chemical, and physical characteristics

Catchment area, discharge, flood frequency, and bed stability varied widely among the 12 streams; however, mean velocity at all sites was similar (Table 1). Table 2A–D summarizes physical, chemical, and biological conditions at each site during NDS incubations. Nutrient concentrations were generally low, although Woolshed and Granity streams had high nitrate levels, possibly because of livestock pastures surrounding the sampling sites. Streamwater N:P ratios suggestive of both N and P limitation were observed. Mean seasonal water temperatures ranged from 3.5 to 14.4°C (winter to summer). The largest number and magnitude of high-flow events occurred during the spring NDS incubations, whereas both winter and summer bioassays were relatively undisturbed.

Seasonal patterns

Strong seasonal differences occurred in both biomass accrual rates and nutrient limitation of biomass accrual. Benthic algal biomass accrual rates (data pooled from all 12 study streams) differed significantly among seasons (p =

TABLE 2. Physical, chemical, and biological parameters measured at the beginning, and hydrological parameters measured during the course of, the nutrient-diffusing substrata (NDS) incubations. AFDM = ash-free dry mass, Q = discharge, %P_c = cellular P content, %N_c = cellular N content, — indicates that no data were collected. Slight discrepancies between nutrient concentrations and N:P ratios are a result of rounding.

Stream	Temperature (°C)	NH_4 (mg/m³)	NO_3 (mg/m³)	PO_4 (mg/m³)	N:P (water)	%N_c (% AFDM)	%P_c (% AFDM)	N:P (cellular)	Maximum Q (no. × median)	No. of d in flood
A: Summer										
West Kowai	18.9	2.9	10.7	0.9	35.4	7.45	0.37	44.6	0.7	0
Kyeburn	—	—	—	—	—	—	—	—	—	—
Bowyers	9.2	0.9	10.8	1.8	14.3	7.53	0.49	34.0	1.5	0
North Kowai	11.7	0.2	10.9	1.0	25.8	8.30	0.30	61.3	0.7	0
Timber	—	—	—	—	—	—	—	—	—	—
Woolshed	13.3	9.7	367.5	3.8	222.7	6.86	0.37	41.1	0.3	0
Victoria	16.9	1.4	2.2	1.7	4.6	6.14	0.70	19.4	2.3	0
Granity	21.0	3.6	249.2	1.4	399.8	3.29	0.51	14.3	20.4	1
Sams	—	—	—	—	—	—	—	—	—	—
Rough	10.7	0.8	21.0	0.8	64.4	7.32	0.50	32.4	1.7	0
Slaty	13.4	1.8	9.9	0.8	32.2	5.27	0.94	12.4	10.6	2
Camp	—	—	—	—	—	—	—	—	—	—
Mean	14.4	2.6	85.3	1.5	99.9	6.52	0.52	32.4	5.4	0.4
SD	4.1	3.1	141.3	1.0	139.8	1.60	0.21	16.7	7.0	0.7
B: Autumn										
West Kowai	8.1	1.7	12.2	1.5	21.2	6.34	0.47	29.7	3.2	1
Kyeburn	10.6	1.1	3.2	1.4	6.7	4.65	0.39	26.4	0.5	0
Bowyers	9.0	1.1	23.9	3.7	15.1	8.75	0.46	42.1	4.3	1
North Kowai	10.0	0.7	5.4	0.9	15.6	6.63	0.38	38.7	2.0	0
Timber	11.8	0.7	10.4	2.7	9.1	8.82	0.62	31.5	0.7	0
Woolshed	11.5	2.0	125.3	5.0	56.9	8.67	0.30	64.0	5.0	3
Victoria	12.1	2.5	2.7	1.6	7.1	5.07	0.50	22.5	3.1	1
Granity	—	—	—	—	—	—	—	—	—	—
Sams	—	—	—	—	—	—	—	—	—	—
Rough	9.8	1.5	10.1	2.2	11.9	4.79	0.63	16.8	2.3	0
Slaty	12.0	2.2	13.4	2.7	13.0	4.85	0.61	17.6	3.9	2
Camp	—	—	—	—	—	—	—	—	—	—
Mean	10.5	1.5	22.9	2.4	17.4	6.51	0.48	32.1	2.8	0.8
SD	1.4	0.7	38.9	1.3	15.5	1.81	0.12	14.7	1.5	1.1
C: Winter										
West Kowai	3.1	2.4	90.0	1.9	108.7	8.22	0.46	39.6	2.7	0
Kyeburn	1.1	2.7	6.7	3.0	7.0	6.87	0.51	29.8	3.8	1
Bowyers	2.7	3.3	12.9	2.7	13.2	6.97	0.32	48.2	1.3	0
North Kowai	3.5	1.7	21.8	1.9	27.3	7.59	0.38	44.2	1.1	0
Timber	2.6	1.6	16.0	1.5	25.8	3.60	0.46	17.3	1.5	0
Woolshed	4.2	1.2	254.2	4.0	143.1	6.91	0.28	54.6	2.3	0
Victoria	2.9	2.3	10.9	1.1	27.6	5.26	0.42	27.7	1.7	0
Granity	4.4	11.1	188.3	1.1	420.3	6.53	0.56	25.8	7.8	6
Sams	—	—	—	—	—	—	—	—	—	—
Rough	2.3	1.5	28.9	0.8	89.6	5.82	0.50	25.8	0.6	0
Slaty	5.0	2.4	27.3	1.3	52.5	4.07	0.52	17.3	5.5	3
Camp	6.2	0.9	37.7	2.0	43.8	3.22	0.76	9.4	1.4	0
Mean	3.45	2.8	63.2	1.9	87.2	5.91	0.47	30.9	2.7	0.9
SD	1.41	2.8	82.7	1.0	118.5	1.67	0.13	14.2	2.1	1.9

TABLE 2.   Continued.

Stream	Tempera-ture (°C)	NH_4 (mg/m³)	NO_3 (mg/m³)	PO_4 (mg/m³)	N:P (water)	%N_c (% AFDM)	%P_c (% AFDM)	N:P (cellular)	Maxi-mum Q (no. × median)	No. of d in flood
D: Spring										
West Kowai	7.5	3.0	22.6	1.1	51.4	6.98	0.56	27.6	2.2	0
Kyeburn	9.6	2.5	4.1	3.1	4.7	6.00	0.85	15.6	5.8	8
Bowyers	8.0	3.2	10.9	2.7	11.6	6.63	0.46	31.9	4.1	1
North Kowai	6.8	2.3	4.7	1.3	11.8	5.57	0.56	22.0	1.8	0
Timber	8.5	2.6	13.8	1.3	27.8	5.32	0.33	35.7	4.7	12
Woolshed	8.5	1.5	99.8	1.6	144.6	8.01	0.48	37.0	5.3	8
Victoria	8.8	2.8	12.1	1.1	30.0	5.44	0.72	16.7	2.5	0
Granity	17.1	6.4	212.1	1.9	261.5	5.01	0.46	24.1	9.8	4
Sams	9.9	4.1	13.0	2.1	18.4	4.04	0.54	16.6	9.9	4
Rough	7.3	2.3	26.2	1.1	60.0	4.07	0.48	18.8	3.5	2
Slaty	9.2	2.7	25.2	1.6	38.5	6.21	1.0	13.8	5.9	3
Camp	—	—	—	—	—	—	—	—	—	—
Mean	9.2	3.0	40.4	1.7	60.0	5.75	0.59	23.6	5.0	3.8
SD	2.8	1.3	62.8	0.7	77.3	1.19	0.20	8.3	2.6	4.0

0.019). Accrual rates were maximum in summer, minimum during winter, and intermediate in both autumn and spring (Fig. 1A). The proportion of streams in which benthic algal biomass was significantly increased by nutrient amendment also differed significantly with respect to season ($p < 0.025$). Nine of 10 streams displayed significant nutrient stimulation (as indicated by NDS bioassays) in summer, whereas only 3 of 11 streams were nutrient-stimulated in winter (Fig. 1B). Both autumn and spring showed intermediate values. The degree of nutrient limitation also displayed a highly significant ($p = 0.003$) seasonal pattern. The overall degree of nutrient limitation was greatest in the summer, lowest in the winter, and intermediate in the autumn and spring (Fig. 1C). The results of individual NDS bioassays are summarized in the Appendix. Invertebrate densities on the NDS also showed significant ($p < 0.001$) seasonal variation; densities were lower in winter than in autumn or spring (Fig. 2). The degree to which invertebrate densities were stimulated by nutrient amendment was also least in winter, but this trend was not significant.

Water temperature was significantly correlated to benthic algal biomass accrual rates and the degree of nutrient limitation (Table 3). Low water temperatures appeared to inhibit benthic algal biomass accrual and reduce the degree of nutrient limitation. At higher temperatures, some bioassays exhibited great rates of biomass accrual and/or a high degree of nutrient limitation, but such responses were not universal; slow biomass accrual and low degrees of nutrient limitation were still observed (Fig. 3). The degree of nutrient limitation was also negatively correlated with streamwater NO_3-N concentrations (Table 3). This pattern appeared to be largely caused by a few instances of strong nutrient limitation in very low-N systems (Fig. 4). Neither benthic algal biomass accrual rates nor the degree of nutrient limitation was strongly correlated with any other variable considered (Table 3).

Method comparison

Streamwater N:P ratios were uncorrelated with cellular N:P ratios of natural periphyton communities (Spearman's rank correlation, $r = 0.089$, $p > 0.5$, $n = 39$) (Fig. 5). Streamwater N:P ratios were poor predictors of which nutrient limited NDS bioassays (Fig. 6A). Although P limitation was only observed at relatively high (~30:1) N:P ratios, N limitation was also observed at high N:P ratios (up to ~400:1). Co-limitation of benthic algal biomass accrual by both N and P occurred over the entire range of streamwater N:P ratios. Similarly, cellular N:P ratios of natural periphyton communities also failed to predict which nutrient limited NDS
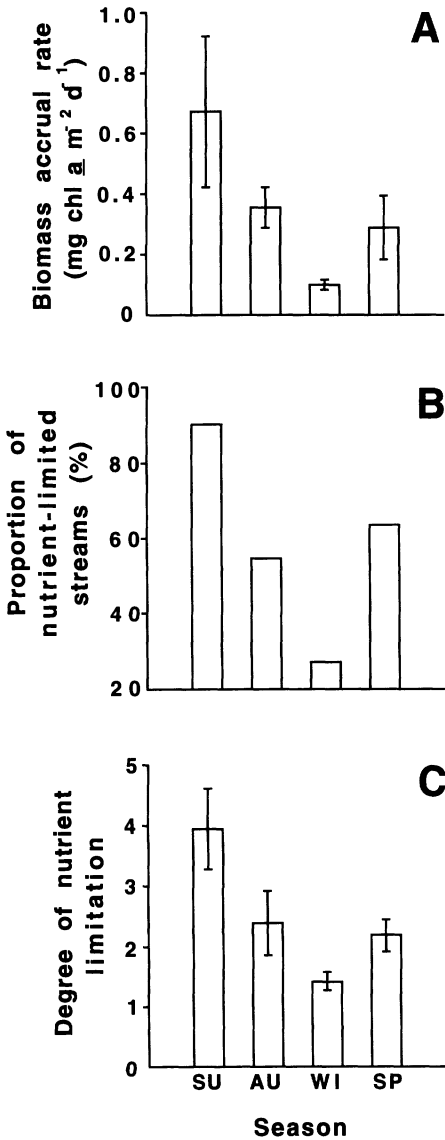
FIG. 1. Seasonal differences in (A) mean benthic algal biomass accrual rates ($p = 0.019$), (B) proportion of streams in which benthic algal biomass increased significantly in response to nutrient amendments ($p < 0.025$), and (C) mean degree of nutrient limitation (ratio of biomass on nutrient-enriched substrata to that on unenriched substrata) ($p = 0.003$). SU = summer, AU = autumn, WI = winter, SP = spring, chl = chlorophyll. Bars are ±1 SE.

lated to which nutrient limited NDS bioassays; limitation of NDS bioassays by N, P, and N + P occurred throughout wide, overlapping ranges of $\%P_c$ and $\%N_c$ values (Fig. 6C, 6D). However, NDS bioassays conducted in streams with severely N-deficient periphyton communities ($\%N_c < 5$) were always N limited.
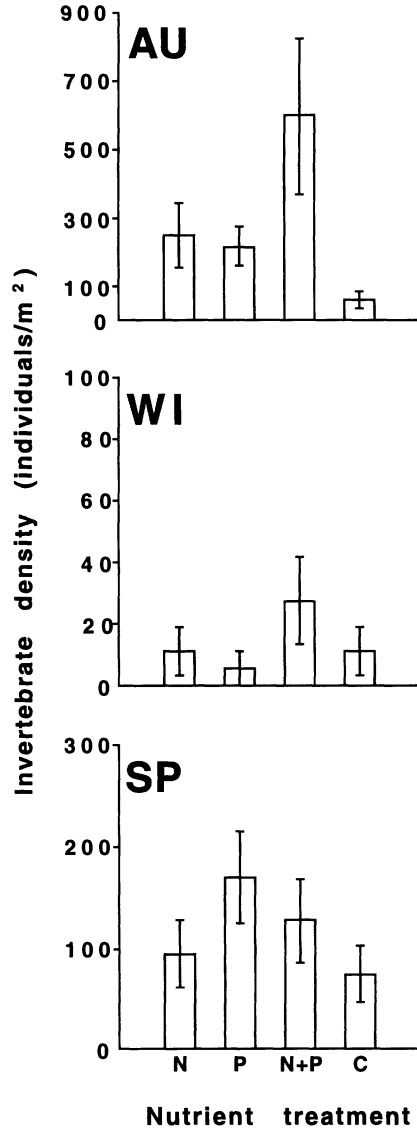

FIG. 2. Mean invertebrate (caddisfly and chironomid) density on each nutrient treatment for 3 seasons ($p < 0.001$ for among-season comparison). Note the change of y-axis scale. AU = autumn, WI = winter, SP = spring. Bars are ±1 SE.

bioassays (Fig. 6B). Nitrogen, P, and N + P co-limitation all occurred over wide ranges of N:P ratios. Cellular nutrient content of natural periphyton communities also was not strongly related to

TABLE 3. Correlation of the rate of benthic algal biomass accrual and the degree of nutrient limitation of benthic algal biomass accrual to measured physical, chemical, and biological parameters ($n = 39$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). AFDM = ash-free dry mass, chl a = chlorophyll a, %P_c = cellular P content, %N_c = cellular N content.

Parameter (units)	Biomass accrual rate	Degree of nutrient limitation
Maximum discharge (multiple of annual median)	0.194	−0.054
Days in flood (no. of d > 3 × annual median)	0.133	−0.110
NH_4-N (mg/m³)	−0.035	0.170
NO_3-N (mg/m³)	0.090	−0.387*
PO_4-P (mg/m³)	0.246	−0.152
Conductivity (μS/cm)	0.112	−0.176
Water temperature (°C)	0.521***	0.463**
%N_c (% of AFDM)	0.165	−0.101
%P_c (% of AFDM)	−0.022	0.196
Chl a on streambed (mg chl a/m²)	0.109	0.160

Discussion

Seasonal patterns

Our results showed seasonal changes in the degree of nutrient limitation of lotic benthic algal community growth. Both the prevalence and the severity of nutrient limitation were greatest in summer, lowest in winter, and intermediate in autumn and spring. These patterns could have been caused by seasonal variation in 5 factors: nutrient supply, disturbance, grazing, temperature, or light. Streamwater nutrient concentrations differed among seasons, and NO_3-N concentrations in streamwater were negatively correlated with the degree of nutrient limitation
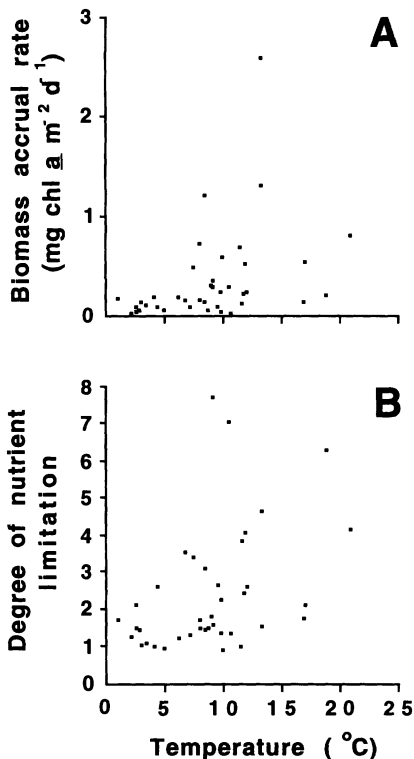

FIG. 3. Relationship of (A) biomass accrual rates and water temperature ($r = 0.521$, $p < 0.001$, $n = 39$), and (B) the degree of nutrient limitation and water temperature ($r = 0.0463$, $p < 0.01$, $n = 39$). chl = chlorophyll.
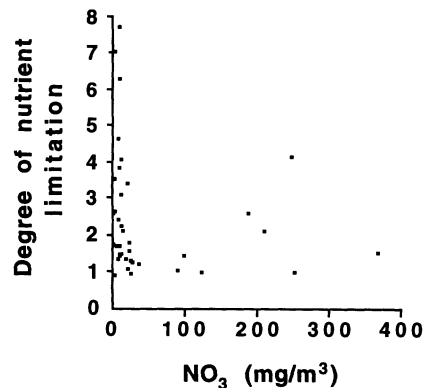

FIG. 4. Scatterplot of the degree of nutrient limitation and streamwater NO_3-N concentration ($r = -0.387$, $p < 0.05$, $n = 39$). Degree of nutrient limitation = ratio of mean biomass on nutrient-enriched substrata to that on unenriched substrata.
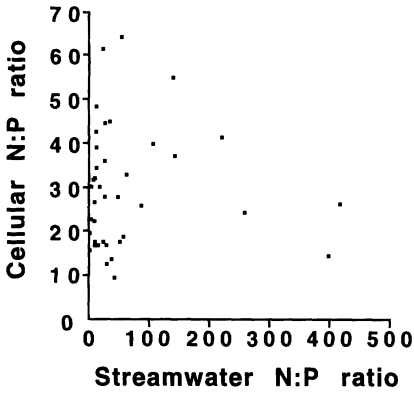
FIG. 5. Scatterplot of streamwater and periphyton cellular N:P ratios ($r = 0.089$, $p > 0.5$, $n = 39$).

of nutrient limitation for all study streams. Rates of benthic algal biomass accrual were not related to streamwater NO_3-N concentrations. Flooding during NDS incubations varied seasonally, but the patterns observed (i.e., the greatest amount of time in flood occurring in spring, but the slowest biomass accrual and least nutrient limitation in winter) indicated that time in flood was not strongly influencing these variables. Differential grazing (either among nutrient treatments or between seasons) could also have influenced our assessment of algal biomass accrual and nutrient limitation. For example, Rosemond et al. (1994) reported that intense grazing prevented seasonal changes in algal biomass in Walker Branch, Tennessee, a 1st-order, temperate, forested stream. In our study, invertebrate densities (and presumably grazing pressure) were lowest in the winter (when algal biomass accrual was slowest). Furthermore, the degree to which invertebrate densities increased on high-nutrient substrata (presumably in response to greater algal biomass on these substrata; e.g., Hill and Knight 1988, Biggs and Lowe 1994) was lowest in the winter (when nu-

of benthic algae. This pattern suggested that seasonality in NO_3-N supply could have influenced the response of algae to nutrient enrichment. However, because the observed correlation was driven by results from just a few sites with low streamwater N and strong nutrient limitation, seasonal differences in N supply were not important in determining the degree
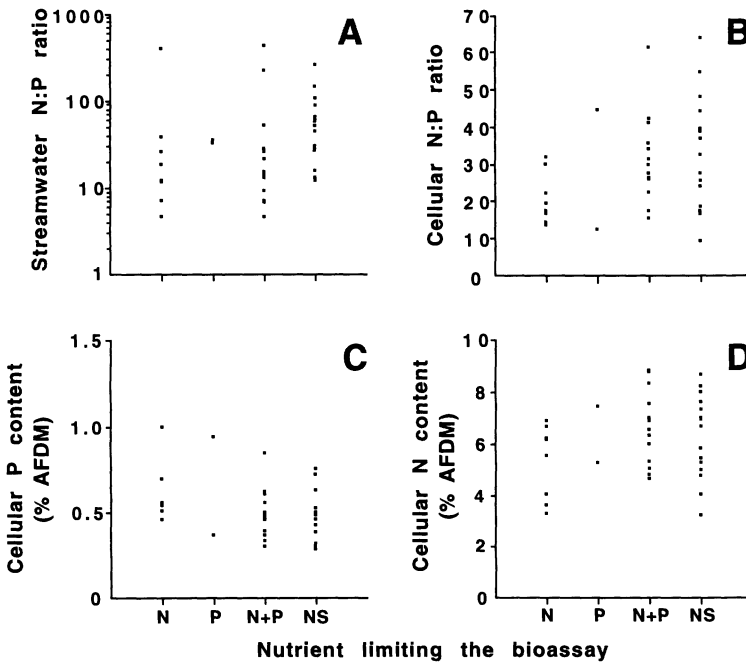

FIG. 6. Results of streamwater N:P ratios (A), cellular N:P ratios of natural periphyton (B), cellular P content of natural periphyton (C), and cellular N content of natural periphyton (D) vs nutrient-diffusing substrata bioassay results. N + P indicates N + P co-limitation, NS indicates non-significant responses to nutrient amendment. AFDM = ash-free dry mass.

trient enhancement of algal biomass was also least). Given these patterns of invertebrate density, grazing probably introduced conservative bias into our results; i.e., without grazing, seasonal differences in algal biomass accrual and nutrient limitation would likely have been even more pronounced.

Water temperature was strongly correlated with both benthic algal biomass accrual rates and the degree of nutrient limitation of algal biomass accrual, suggesting that physiological responses to temperature (i.e., slower growth and reproduction in cold water, see DiNicola 1996) could be the underlying causal mechanism for the observed seasonal patterns. However, other factors known to influence algal growth (e.g., light) are likely to covary with water temperature. Thus, as in any correlation-based analysis, conclusions about the causal influence of temperature must be tentative. In a year-long study at a location climatically similar to our study area, Bothwell (1988) found that water temperature was more highly correlated with, and explained more of the variance in, benthic algal growth rates in outdoor, artificial streams than did light. Similar manipulative experimentation will be required to provide a definitive answer to this question in our systems.

Four other studies have investigated year-round, in-situ responses of periphyton to nutrient enrichment. In contrast to our findings of strong seasonality, NDS bioassays conducted in the Upper Guadalupe River, Texas, indicated nearly constant levels of nutrient limitation throughout the year (Stanley et al. 1990). A probable explanation for this discrepancy is the difference in climate. The annual range of water temperature in the Upper Guadalupe River was 10 to 30°C, whereas in our streams, temperatures ranged from 1.1 to 21.0°C. In 2 streams climatically similar to those in our study (annual water temperature range: <4 to 17°C and 4 to 18°C, respectively), Biggs et al. (1998a) and Winterbourn (1990) reported that the degree of nutrient limitation varied among seasons, with a minimum in winter. Similarly, Allen and Hershey (1996) also reported significant seasonal differences in nutrient limitation of benthic algal community biomass in a Minnesota stream (annual temperature range: 4 to 22.5°C). Taken together, these results indicate that seasonal differences in ability of nutrient additions to stimulate biomass accrual may be a general phe-

nomenon of temperate stream systems, and suggest that this response may be mediated by temperature.

A common goal of NDS bioassay experiments is to determine whether benthic algal community biomass in a stream is nutrient limited and, if so, which nutrient is limiting (e.g., Grimm and Fisher 1986, Lowe et al. 1986, Hill and Knight 1988). Our study indicates that such results should be applied cautiously once stream conditions have shifted away from those under which the study was conducted. Even if the same nutrient limits algal growth during different seasons, the magnitude of the response to nutrient loading may differ substantially.

Our results also suggest that benthic algae have the potential to impose a seasonal pattern on whole-stream nutrient transport. If seasonality in nutrient limitation of benthic algal biomass accrual is caused by reduced growth and sequestering of nutrients by benthic algae during colder periods, then a given quantity of a nutrient should move further downstream per unit time in winter than in summer (i.e., nutrient spiraling length will differ seasonally). Increased spiraling length would increase nutrient loss rates of a system (see Mulholland 1996). In Walker Branch, Mulholland et al. (1985) found seasonal differences in PO_4-P uptake by periphyton, with the greatest uptake rates occurring during periods most favorable for algal growth. However, changes in periphyton uptake of PO_4-P did not alter P spiraling length, because of the much greater influence of PO_4-P uptake by heterotrophic communities on coarse and fine particulate organic matter (C/FPOM). In systems without large amounts of C/FPOM (e.g., large gravel-bed rivers), seasonal differences in periphyton metabolism may strongly influence nutrient spiraling lengths.

Rates of biomass accrual displayed significant seasonal variation. The greatest rates occurred in summer, the lowest rates in winter, with intermediate rates in autumn and spring. A winter minimum in accumulation rates of lotic benthic algal biomass appears to be a general pattern in temperate streams (Butcher 1946, Stockner and Shortreed 1976, Gale et al. 1979, Lowe and Gale 1980, Cox 1990b, Biggs et al. 1998a); however, Biggs (1988a) reported higher net rates of biomass accrual in autumn and winter in 9 New Zealand rivers, and data from Winterbourn (1990) indicate a lack of seasonality in an-

other New Zealand stream. As a whole, the data indicate that many (but not all) temperate streams show seasonal patterns of benthic algal biomass accrual. Seasonality of biomass accrual rates has implications for stream function. In addition to influencing nutrient spiraling (see above), recovery from localized disturbances (e.g., small-scale removal of algal biomass caused by activity of aquatic or terrestrial animals, see Peterson 1996) should occur much more quickly in summer than in winter.

A concurrent study documented seasonal variation in the productivity of natural benthic algal communities at these sites. Algal productivity was greatest during the summer, intermediate in autumn, and least in spring and winter (Biggs et al. 1999). The similarities of the seasonal patterns in the productivity of natural algal communities and of algal biomass accrual rates on NDS suggests that seasonal differences in algal metabolic activity played an important role in algal biomass accrual on NDS.

Biomass was only measured at the end of the bioassay incubations, so it was impossible to calculate accrual rates using an exponential growth model (e.g., growth rates of Stevenson 1996). Estimates of the exponential growth rate for each bioassay produced by assigning a low, non-zero value (0.001 mg chlorophyll a/m^2) to day 0 biomass are highly correlated to linear accrual rates (Spearman's rank correlation, r = 0.774, $p < 0.001$, $n = 39$). The logarithmic estimates display highly significant seasonal differences identical to those of linear estimates, and correlations between the logarithmic estimates and the physical, chemical, and biological variables listed in Table 3 are essentially the same as those derived using linear estimates. Thus, the choice of model used to calculate accrual rates does not appear to alter the overall results of the study.

Method comparison

Streamwater and cellular N:P ratios did not covary. Therefore, both ratios could not have been simultaneously depicting algal nutrient status, and some factor(s) (e.g., N and P present in non-algal components of the periphyton, temporal variability in streamwater nutrient concentrations) was decoupling these ratios.

The identity of the nutrient that limited NDS bioassays could not be predicted from inspec-

tion of streamwater nutrient ratios. Although P limitation only occurred under high (~30:1) N: P ratios, N limitation occurred over a wide range of N:P ratios (from 4:1 to 400:1), as did N + P co-limitation. Many of the observed N:P ratios lay between 22 and 44, a range in which Schanz and Juon (1983) also found that streamwater N:P ratios did not accurately reflect which nutrient (N or P) limited algal growth. At N:P ratios <22, Schanz and Juon (1983) found that N was always the nutrient limiting algal growth. In this study, N limitation was common at streamwater N:P ratios <20:1, but N + P co-limitation was also frequently observed at similar N:P ratios. Cellular nutrient ratios of natural periphyton also were poor predictors of NDS bioassay results. A wide range of cellular N:P ratios was associated with N, P, and N + P co-limitation of NDS bioassays.

Growth limitation of *Cladophora* by N or P occurs when cellular nutrient content drops below threshold values of 11% N and 0.5% P, respectively (Gerloff and Fitzgerald 1976, Auer and Canale 1982). Biggs et al. (1998a) found that these threshold values of %N$_c$ and %P$_c$ of natural periphyton communities in the Kakanui River, New Zealand, were generally consistent with which nutrient limited biomass accrual on NDS bioassays. In our study, agreement between cellular nutrient content of natural periphyton communities and NDS bioassays was more limited. Nitrogen, P, and N + P co-limitation of NDS bioassays was generally associated with wide, overlapping ranges of %N$_c$ and %P$_c$. However, NDS bioassays were always N limited in streams with periphyton communities strongly deficient in N (%N$_c$ < 5).

Some field studies have found streamwater N: P ratios, either DIN:SRP or total N to total P to be good predictors of periphyton nutrient status (e.g., Grimm and Fisher 1986, Hill and Knight 1988, Lohman et al. 1991, Peterson et al. 1993). Other field studies have failed to find such a relationship (Kutka and Richards 1997), or have shown responses opposite to those predicted from N:P ratios (Allen and Hershey 1996). In some cases, the lack of relationship between N: P ratios and nutrient stimulation of benthic algal biomass may be a result of very short incubation times (e.g., Kutka and Richards 1997); however, Allen and Hershey (1996) used an adequate (3 wk) incubation period. Our results support Allen and Hershey's (1996) conclusion that

"... DIN:SRP ratio is not a reliable indicator of nutrient limitation ...".

In chemostat culture systems, the N:P ratio of the environment (i.e., the culture medium) is an excellent predictor of which nutrient limits the biomass of a single algal species (e.g., Chiaudani and Vighi 1974, Rhee 1978). One possible cause for the lack of predictive power of streamwater N:P ratios in our study is that the response variable was community biomass, not the biomass of a single species. Algal species differ in their nutrient requirements and optimum N:P ratios (e.g., Rhee and Gotham 1980, Tilman et al. 1982, Borchardt 1996). Thus, in a multispecies algal community, different species are likely to be limited by different resources, including different nutrients. Therefore, the concept of single-nutrient limitation does not strictly apply to communities, and should only be used as a convenient shorthand, if at all (see Tilman et al. 1982, Borchardt 1996). Limitation of some species by N and others by P should result in unpredictability of whether N or P limits community biomass within a range of intermediate N:P ratios. Schanz and Juon (1983) suggested that the multispecies nature of their study communities contributed to the indeterminacy of predictions of N or P limitation at N:P ratios between 22:1 and 44:1. A similar phenomenon is likely to have occurred in our study.

A 2nd possibility for the discrepancies between streamwater N:P ratios and NDS bioassay results is that the streamwater DIN and SRP concentrations did not reflect actual nutrient availability. Analyses based upon other fractions of the N and P pools have provided better predictive ability in both benthic (Dodds et al. 1997) and planktonic (Morris and Lewis 1988, Axler et al. 1994) systems. Alternatively, factors such as heterogeneity in nutrient availability within periphyton communities and nutrient cycling within the periphyton matrix (e.g., Mulholland 1996, Wetzel 1996) may have obscured relationships between streamwater nutrient ratios and nutrient limitation of benthic algal biomass accrual.

The time lag between measurement of streamwater N:P ratios and obtaining bioassay results inherent in our study design may have also contributed to the lack of predictive power of streamwater N:P ratios. Streamwater N:P ratios were based on water samples collected at NDS deployment, whereas bioassays integrated nutrient conditions over the period of incubation. Streamwater nutrient concentrations at some sites displayed a great deal of variability (Biggs et al. 1999). More detailed temporal assessment of streamwater N:P ratios may reveal a stronger relationship between streamwater nutrients and NDS bioassay results.

Similarly, cellular N:P ratios and nutrient content of natural periphyton communities may not have accurately depicted the nutrient status of algae grown on the NDS bioassays. The contribution of non-algal components of the periphyton to cellular N and P measurements could have been an important source of error. Although non-algal N and P did not appear to cause serious inaccuracies in earlier studies of similar systems (Biggs and Close 1989, Biggs 1995, Biggs et al. 1998a), it is a well-known problem in planktonic systems (e.g., Morris and Lewis 1988), and certainly occurs in benthic measurements (Kahlert 1998). Had we examined the cellular nutrient ratios or cellular nutrient content of the algae grown on the NDS, the relationship between cellular nutrients and bioassay results may have been much stronger. The time lag phenomenon discussed above may have also reduced the predictive power of cellular nutrient ratios and cellular nutrient quotas. Based on a review of the literature, Kahlert (1998) considered cellular N:P ratios of >32 to indicate P limitation of freshwater benthic algae, and cellular N:P ratios <12 to indicate N limitation. In our study, most cellular N:P ratios associated with either N- or P-limited bioassays were between 32 and 12; thus, our data neither support nor contradict this generalization.

Limited statistical power may have caused the prevalence of N + P co-limitation to be overestimated. It is possible that some communities exhibited small, (but real) increases in biomass as a result of the addition of a single nutrient (e.g., N) with further increases in biomass prevented by secondary limitation by another nutrient (e.g., P). When both nutrients were supplied in combination, such communities could have displayed greatly increased biomass. Small biomass increases resulting from single nutrient addition may have been statistically undetectable, but the much greater responses to addition of both nutrients are more likely to have been statistically significant. In such cases, the community would have been erroneously classified as co-limited by N + P. The possibility of not

detecting real differences (i.e., type II error) is common to all uses of inferential statistics, and can be reduced by increased replication. Increased replication might have improved our ability to resolve some apparent cases of N + P co-limitation into either N or P limitation, thereby improving the agreement between experimental results and the inferences drawn from nutrient ratios or cellular nutrient content. For example, if the N + P co-limited communities grown at low and high streamwater N:P ratios were shown (via increased replication and greater statistical power) to actually be N and P limited, respectively, then experimental results would validate N:P ratio predictions. In some cases, improved ability to classify communities as either N or P limited would not increase the agreement between predictions and experimental results because of the overlapping ranges of nutrient ratios and cellular nutrient content associated with bioassays already identified as being limited by either N or P.

Non-significant responses of algal biomass to nutrient amendment were observed over wide a wide range of N:P ratios and cellular nutrient content. This result is to be expected; N:P ratios provide no information as to whether nutrients are limiting, they only suggest which nutrient (N or P) may be limiting. Similarly, without information about the importance of other potentially limiting factors, cellular nutrient content cannot be used to predict whether nutrients are limiting.

In conclusion, rates of benthic algal biomass accrual and the magnitude of additional growth as a result of nutrient amendment varied seasonally. Biomass accrual rates and nutrient stimulation of growth were greatest in the summer, least in the winter, and intermediate in spring and autumn. The discordance observed among several methods commonly used to infer which nutrient (N or P) is potentially limiting to algal biomass demonstrates that they are not universally interchangeable. Each technique has its assumptions and limitations; some accurately reflect algal nutrient status under a narrowly circumscribed set of conditions. Choosing the technique appropriate for a given purpose is critical.

Acknowledgements

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Appendix

Results of individual bioassay experiments using nutrient-diffusing substrata. Treatments: N = nitrogen, P = phosphorus, N + P = nitrogen and phosphorus, C = control. *p*-value refers to the overall significance of the individual experiment. NS = none of the treatments differed significantly. Within each individual experiment, treatments marked with the same letter are not significantly different. Bars are ±1 SE. chl = chlorophyll.
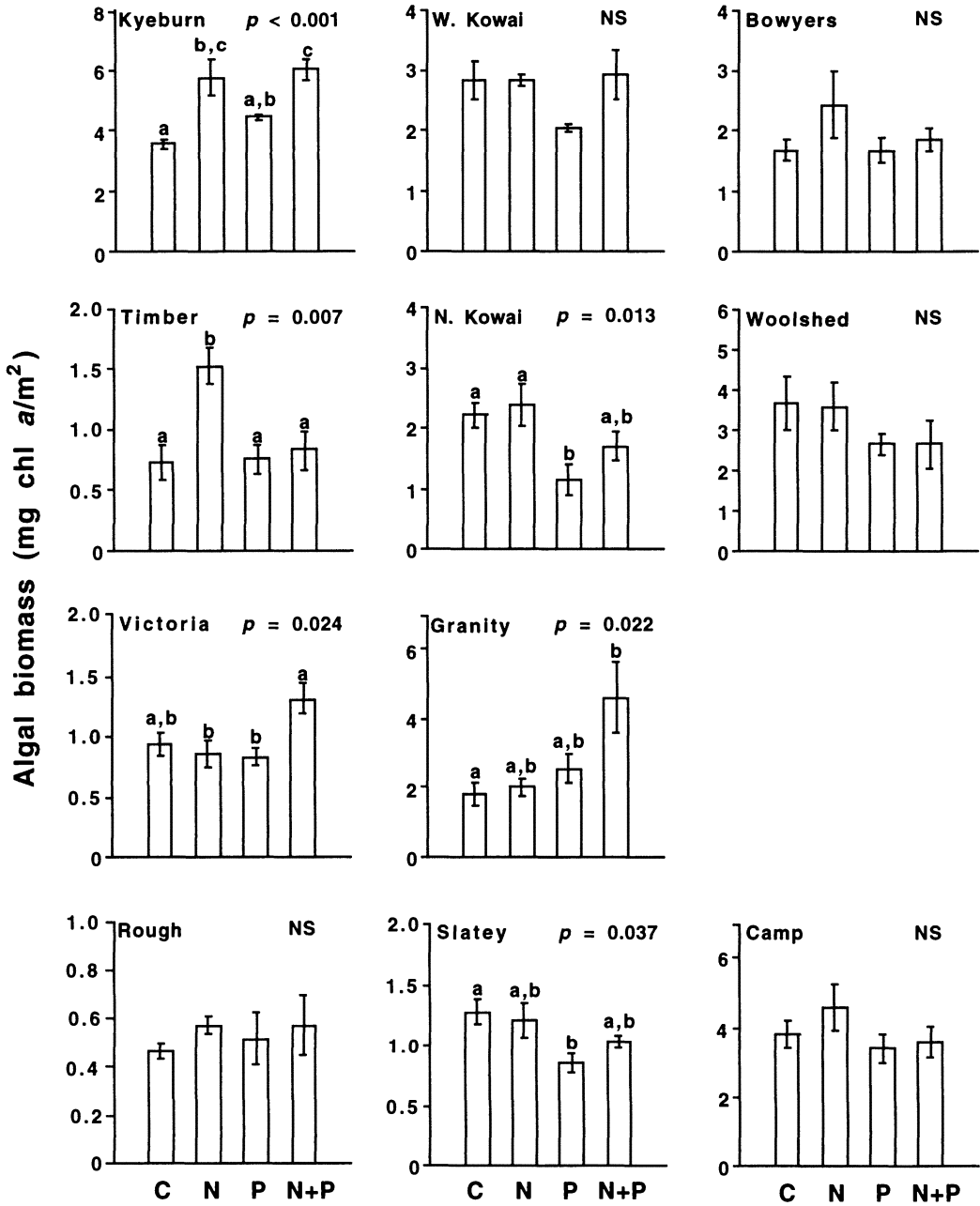
Summer

Algal biomass (mg chl a/m^2)

Nutrient treatment

Autumn

Algal biomass (mg chl *a*/m^2)
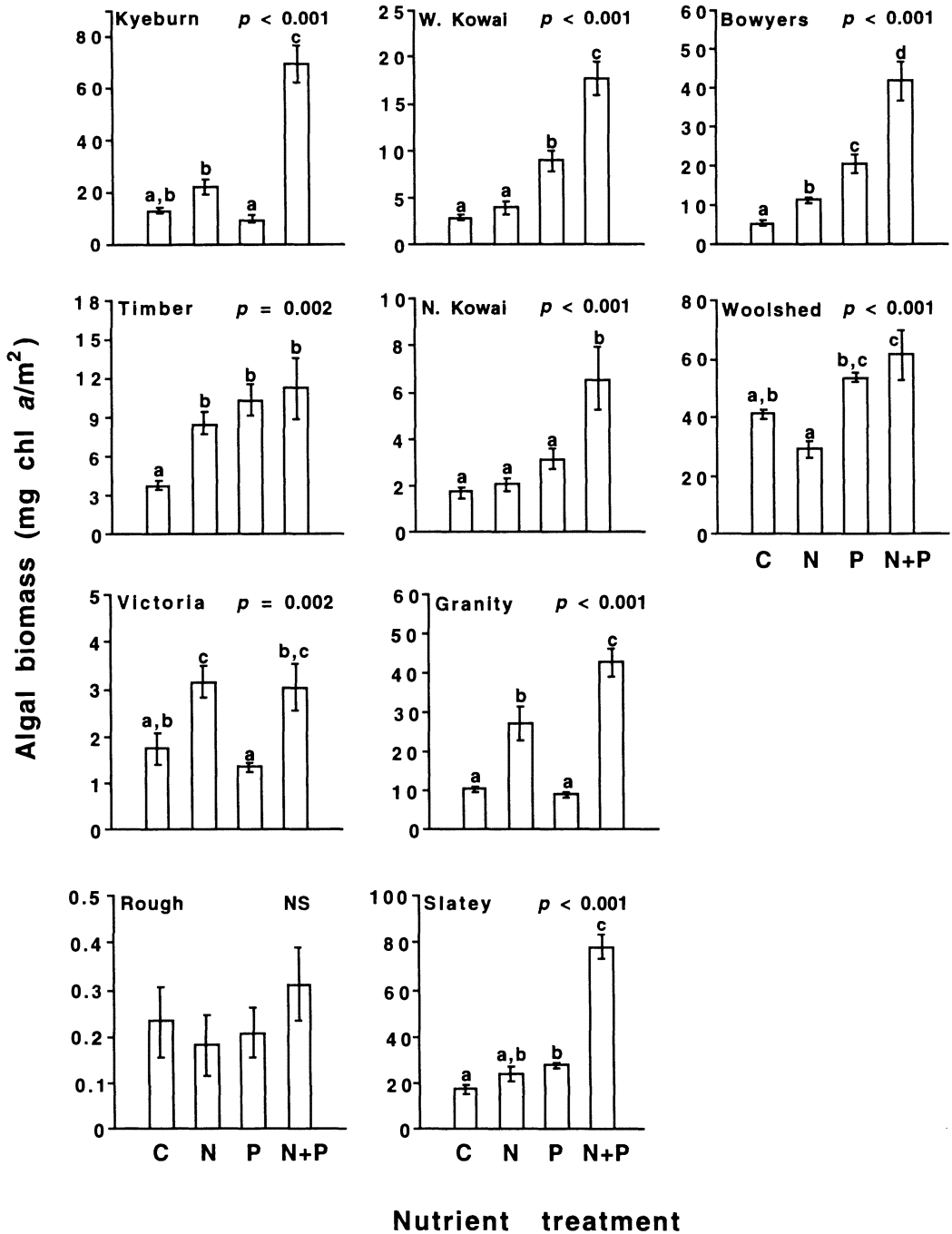
Nutrient treatment
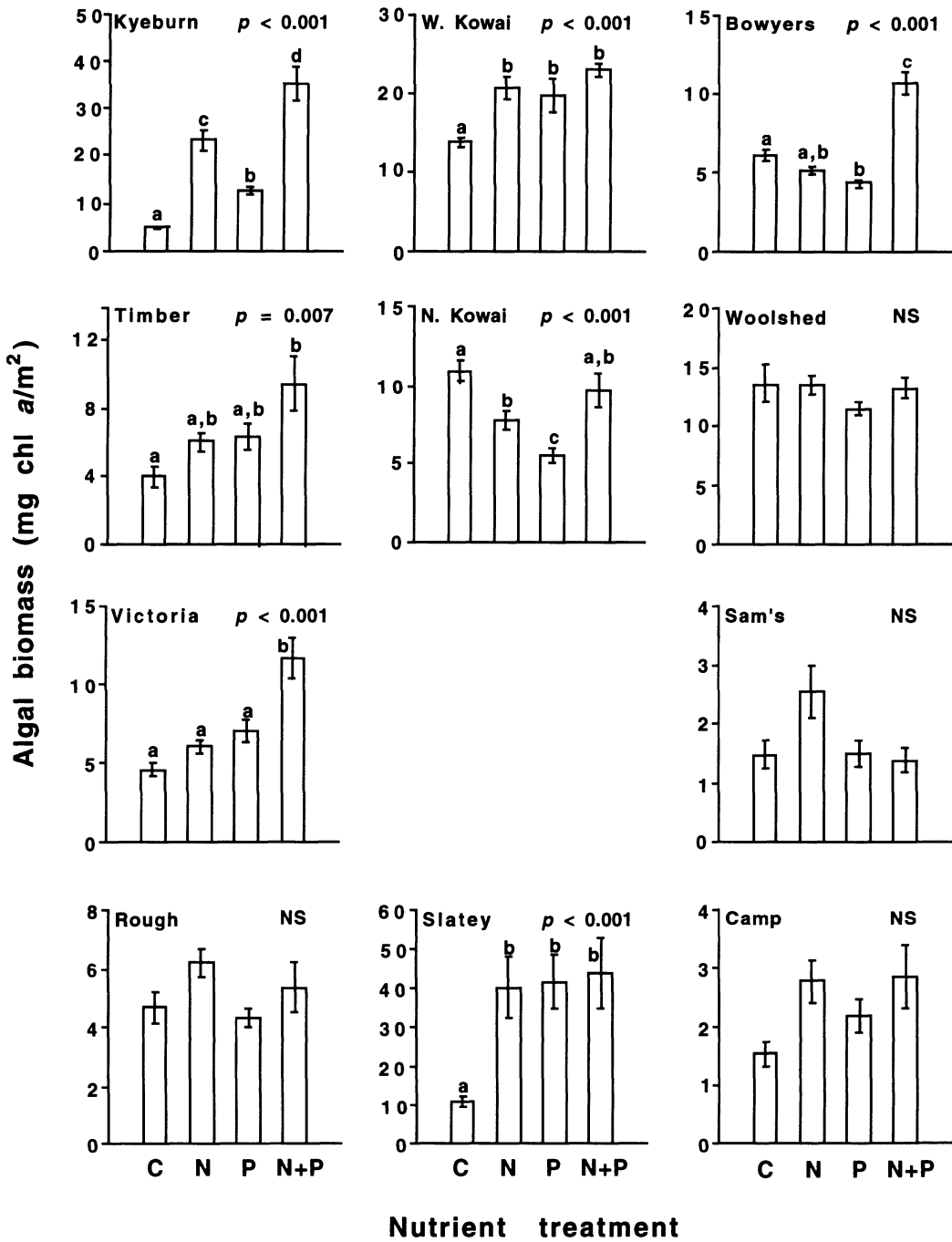
Winter

Nutrient treatment

Spring

Algal biomass (mg chl a/m^2)

Nutrient treatment