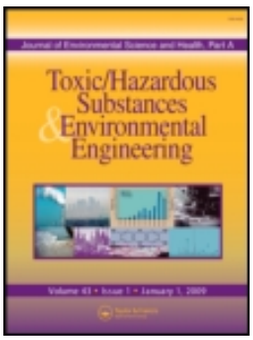

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Spatial and Temporal Variabilities of Nutrient Limitation Based on In Situ Experiments of Nutrient Enrichment Bioassay

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ABSTRACT

In situ experiments of Nutrient Enrichment Bioassays (NEBs) were performed in a morphologically complex reservoir of a temperate region to identify primary limiting nutrient regulating phytoplankton productivity and determine a severeity of the nutrient limitation. Absolute nutrient contents and nutrient ratios of TN : TP and TDN : TDP in the ambient water indicated a potential limitation of phosphorus. This outcome agreed with the results of NEBs; Algal response in the P treatments, expressed as a ratio of $CHL_f : CHL_i$, showed significantly ($p < 0.05$) greater algal response than P + NH_4-N or P + NO_3-N treatments. The magnitude of the limitation, however, showed large spatial and temporal variations. The response in treatments enriched with phosphorus (P, P + NH_4-N, and P + NO_3-N) was greatest in the downlake zone and least in the point-source zone, while the response was greater during summer monsoon than any other seasons. Algal growth rate experiments showed that the response in treatments enriched with NO_3-N and P + NO_3-N never exceeded $> 0.50\,\mu g\ L^{-1}$ per day and was significantly ($p < 0.05$) less than that in the three treatments

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with P (1P, 2P, and $P + NH_4$-N), indicating a reduced response in the treatments enriched with NO_3-N. The suppression of nitrate on algal growth seemed to be involved with inhibition effect of nitrogenase activity through low-nitrate uptake rate in the high nitrate-rich environment and large additions of potassium with phosphorus in spike. Regression analysis of $\log_{10}$-transformed $CHL_f : CHL_i$ ratios against ambient nutrient contents showed that in situ algal response in the P treatments was inversely related ($r = -0.77$; $p < 0.001$) with ambient TP and positively correlated ($r = 0.75$; $p < 0.001$) with TN : TP ratios. This result suggests that in situ response depends largely on external loading of limiting nutrients.

Key Words: Nutrient limitation; Nutrient enrichment bioassay; Seasonality; Algal growth.

INTRODUCTION

Short-term nutrient enrichment bioassay techniques in lentic systems have been used as a key tool to identify which elements regulate phytoplankton growth or productivity.[1,2] This approach is based upon Leibig's minimum law that one or two elements can limit biotic growth under steady-state conditions[3] and has significant implications for efficient lake managements and primary nutrient controls from various watersheds. Previous numerous researchers[4–8] pointed out that phosphorus is often considered the most important limiting nutrient controlling the lake productivity of temperate, inland waters. However, recent comprehensive literature reviews, based on nutrient bioassay experiments in lakes of North Ameria[9] demonstrated that nitrogen in lentic systems had a more important role as a limiting factor than previously recognized.

An identification of nutrient limitation in lentic systems may not so simple due to morphological heterogeneities of waterbodies and seasonal hydrodynamic characteristics.[10] Recently, nutrient models of reservoirs suggested that the types of exported nutrients (N vs. P) and the magnitude of nutrient loading, as a factor controlling lake trophic state, differ along the main axis from the headwater to downlake reach. These situations produce spatial variations in the response of algal growth to ambient nutrient concentrations.[11] In spite of this fact, many researches to identify the limiting nutrient were frequently conducted in a single location, especially, near the dam.

In this study, in situ nutrient bioassay experiments were performed in a morphologically complex reservoir of a temperate region (Taechung Reservoir, Korea) between premonsoon 1994 (June) and postmonsoon 1994 (September). The potential nutrient limitation is demonstrated in the study using several indicators of ambient nutrient ratios of dissolved and total nitrogen and phosphorus, in situ experiments of nutrient enrichment bioassays (NEB), and algal growth rate experiments. This research provides valuable information for efficient lake managements and eutrophication controls.

MATERIALS AND METHODS

Sampling Locations and Data Presentation

This study was conducted in a morphologically complex reservoir (Taechung Reservoir), which is located in the middle part of the Korean peninsula (36°50′ N, 127° 50′E) and has dendritic shape in the basin morphology. The selection of sampling sites in Taechung Reservoir was based on the morphometry along the longitudinal axis and the position of external nutrient loads to the reservoir. As shown in Fig. 1, samples were collected from headwater (S1), mid-lake (S2), and downlake zones (S3) along with point-source area (S4, a location receiving sewage effluent and fish-farm wastewater). Terms of premonsoon (PRE; January–June),

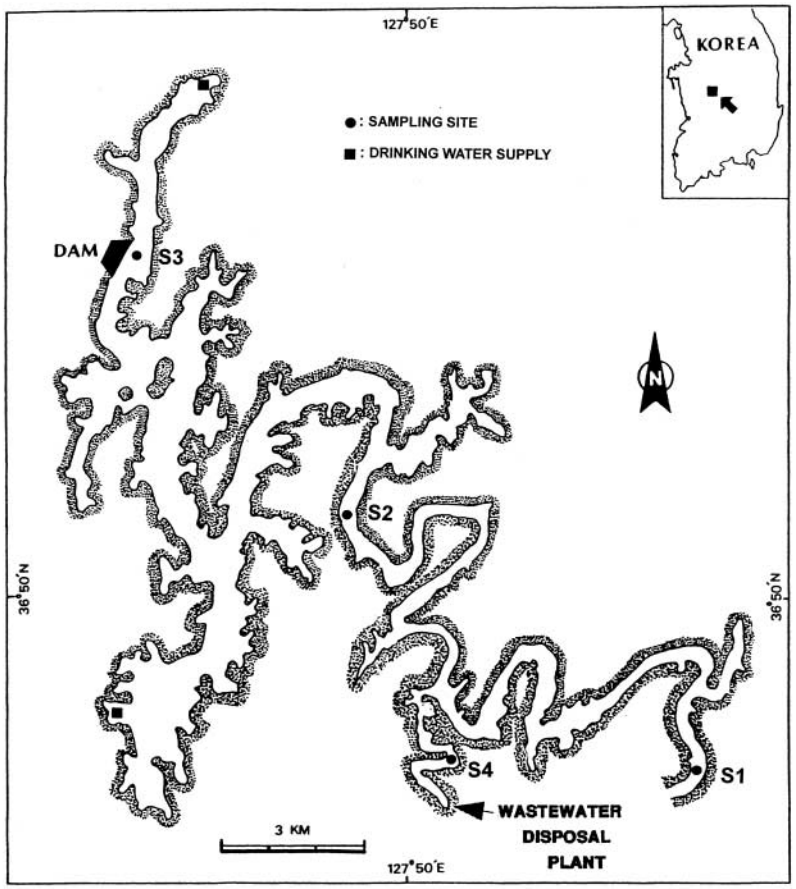

Figure 1. Locations conducted the Nutrient Enrichment Bioassays (NEBs). Characters of S1, S2, S3, and S4 indicate the headwater, mid-lake, downlake, and point-source zone, respectively.

monsoon (MON; July–August), and postmonsoon (POS; September–December) were used in describing temporal conditions.

Chemical Analysis

Secchi transparency (20 cm disk), and temperature (YSI Model 51B meter) were measured at the time of sample collection. Water samples were covered to prevent exposure to direct sunlight, stored in ice, and analyzed in the laboratory within 12–36 hours as follows. Total nitrogen (TN) and total dissolved nitrogen (TDN) were measured by second derivative method after a persulfate digestion.[12] Total phosphorus (TP) and total dissolved phosphorus (TDP) were determined using the ascorbic acid method after persulfate oxidation.[13] Soluble reactive phosphorus (SRP), ammonia nitrogen (NH_4-N) and nitratenitrite nitrogen (NO_3-N), were measured by APHA methods (14). Chlorophyll (CHL) concentration was measured by using a spectrophotometer (Bechman Model DU-65) after extraction in hot ethanol.[15] Nutrient analyses were performed in triplicate; suspended solids and CHL were measured in duplicate.

Experimental Design of Nutrient Enrichment Bioassays (NEBs)

The experiments of NEBs followed the approach of nutrient stimulation employing *in situ* polyethylene cubitainers[2,16] The field setting of NEBs was conducted in the headwater zone (S1), midlake zone (S2), downlake zone (S3), and the point-source zone (S4) during May–September. This experiment was to determine whether algal response to nutrient additions varied spatially and temporally at four different sites along the length of the reservoir and three different depths. Surface water was mixed in a 120 L polyethylene-lined container and dispensed into 10 L translucent polyethylene cubitainers (Nalgene company). Unfiltered surface water was suspended at one or three depths and incubated 5–6 days in cubitainers. The experimental design included a control (C, no nutrient additions), two different nitrogen additions (NH_4-N and NO_3-N treatments), a phosphorus addition (P treatment), and two different simultaneous enrichments of nitrogen and phosphorus ($P + NH_4$ and $P + NO_3$ treatments). Potassium phosphate (K_2HPO_4) was used to spike phosphorus treatments and potassium nitrate (KNO_3) and ammonia chloride (NH_4Cl) stock solutions were used as sources of NO_3-N and NH_4-N, respectively.

Also, growth rate experiment near the dam was conducted to compare algal response over time to two forms of N along with P additions. Treatments were spiked with 1/2, 1, and 2 times ambient P concentrations. Duplicate 250 mL subsamples were serially taken from each cubitainer on 2, 5, 8, 11, and 14 June to determine maximum algal growth at a given P concentration. The experiment included: Control (C), NH_4-N ($25\,\mu g\,L^{-1}$), P ($4.1\,\mu g\,L^{-1}$), 2P ($8.2\,\mu g\,L^{-1}$), 4P ($16.4\,\mu g\,L^{-1}$), $1P + NH_4$-N ($4.1\,\mu g\,L^{-1}$ P plus $25\,\mu g\,L^{-1}$ NH_4-N), and $1P + NO_3$-N ($4.1\,\mu g\,L^{-1}$ P plus $400\,\mu g\,L^{-1}$ NO_3-N) treatments. Stock solutions for the spikes were same as above experiments.

In the NEBs, the response of phytoplankton to the treatments was determined by measuring chlorophyll (CHL). The results were expressed as the ratio of final chlorophyll (CHL_f) to initial chlorophyll (CHL_i) or ratios of final CHL in each treatment to CHL in the control. Differences among treatments were analyzed by one-way ANOVA Duncan's multiple range test.[17]

RESULTS AND DISCUSSIONS

Nutrient Conditions

During the experiments of NEBs, TP in the ambient water showed a large variation depending upon the season and location. Mean TP, based on all sites by season, was $31\,\mu g\,L^{-1}$ and TDP was a half of the TP, while mean TN was $1.55\,mg\,L^{-1}$ and mean TDN was $1.188\,mg\,L^{-1}$ (Table 1). Trophic state of TN and TP, based on the criteria of Nurnberg,[18] was hypertrophic and eutrophic, respectively. Dissolved inorganic nitrogen (DIN) accounted for some 70% of TN and a large fraction of the TDN was inorganic. More than 90% of DIN was NO_3-N, whereas mean levels of NH_4-N was composed of $<5\%$ of DIN (Table 1), indicating a dissolved nitrogen-rich system. Seasonal mean TP, based on all sites for NEBs, did not highly vary with season (26, 35, and $32\,\mu g\,L^{-1}$ in the PRE, MON, and POS, respectively) and TDN values were greater than $0.95\,mg\,L^{-1}$ regardless of the season. All fractions of phosphorus and nitrogen were greater in the site of point-source than any other sites of the reservoir. Measurements in the mainstem axis of the reservoir

Table 1. Fractions of nitrogen and phosphorus in the ambient epilimnetic water during the Nutrient Enrichment Bioassays (NEBs).

		Nitrogen[a]							Phosphorus[a]	
Season	Site	TN	TDN	NO_3-N	NH_4-N	DIN	TN:TP	DIN:TDP	TP	TDP
PRE	H	1.60	1.57	1.18	0.04	1.22	62	102	26	12
	M	1.58	1.36	1.27	0.01	1.28	69	116	23	11
	D	1.29	1.08	0.90	0.01	0.91	185	182	7	5
	P	1.69	1.26	1.05	0.00	1.05	35	66	48	16
MON	H	2.25	ND	2.06	0.08	2.14	48	ND	47	ND
	M	1.91	ND	1.24	0.06	1.29	44	ND	43	ND
	D	1.36	ND	0.87	0.03	0.90	139	ND	6	ND
	P	2.16	ND	1.77	0.03	1.80	48	ND	45	ND
POS	H	0.95	0.87	0.41	0.09	0.51	27	46	35	11
	M	1.24	1.10	0.62	0.14	0.76	46	63	27	12
	D	1.28	1.14	0.81	0.06	0.86	107	144	12	6
	P	1.32	1.12	0.56	0.04	0.59	23	35	57	17

PRE = premonsoon, MON = Monsoon, and POS = Postmonsoon;
H = Headwater zone, M = Midlake Zone, D = Downlake Zone, P = Point-Source Zone.
[a]Units for the nitrogen and phosphorus are $mg\,L^{-1}$ and $\mu g\,L^{-1}$, respectively.

showed that TP, TDP and PP declined >3 fold from the headwater zone (range: 26–47 $\mu g\,L^{-1}$) to the downlake zone (6–12 $\mu g\,L^{-1}$) along the axis of the reservoir and that TN:TP ratios increased >2 fold from the headwater zone (range: 27–62) to the downlake zone (107–185). Based on these facts, longitudinal gradients in the nutrient contents and its ratios were evident in this system. Overall, mass ratios of TN:TP and TDN:TDP were 50 and 108, indicating that a potential phosphorus limitation, based on the criteria of Fosberg and Ryding.[19]

Spatial Variation of NEBs

Algal response to nutrients was expected to vary among locations due to longitudinal gradients in ambient values. At all sites, CHL yields in P treatments were significantly ($p < 0.05$) greater than the control or nitrogen treatments, and response in the control was greater than N treatments (Table 2). Among experiments conducted during June–September, phosphorus limitation was consistent regardless of the location, but the magnitude of growth response to P, corrected for growth in the controls varied spatially. During premonsoon season, at four locations phosphorus alone or in combination with N produced a significantly ($p < 0.05$) greater algal response than the control (C), NO_3-N or NH_4-N treatments (Table 2). The magnitude of algal response, however, varied among locations. Algal growth in the P treatment was greatest at the headwater and downlake sites where increases were >100% relative to the controls (Fig. 2). Treatment of NO_3-N did not differ from controls at the headwater and mid-lake, but was significantly ($p < 0.05$) less than controls at the point-source and downlake locations (Table 2).

Table 2. Results of Nutrient Enrichment ioassays (NEBs) at all zones during three seasons[1].

				Ratios of $CHL_f : CHL_i$						
Season	Site	Z (m)	t (°C)	C	NO	NH	P	P+NO	P+NH	ANOVA test
PRE	H	1.0	24.0	1.13	0.98	0.98	2.48	2.16	2.56	P= P+HH > P+NO > C+NH=NO
	M	1.0	22.5	1.23	1.13	1.19	1.73	1.44	1.97	P+NH > P > P+NO > C=NH+NO
	D	2.7	22.0	1.17	0.82	1.20	2.54	1.26	2.83	P+NH > P > P+NO=NH > C > NO
	P	0.5	23.8	1.03	0.92	0.91	1.63	1.51	1.73	P+NH > P > P+NO > C > NO=NH
MON	H	0.6	27.4	1.04	1.00	1.25	0.70	1.27	1.35	P > P+NH > P+NO > NH > C > NO
	M	0.7	27.1	0.98	0.86	0.91	1.54	1.37	1.37	P > P+NO=P+NH > C > NH=NO
	D	2.3	25.5	1.15	1.07	1.02	6.00	5.54	5.45	P > P+NO=P+NH > C > NH=NO
	P	0.5	28.5	1.01	0.89	1.06	1.64	1.06	1.38	P > P+NH > P+NO=NH > C > NO
POS	H	0.7	28.6	1.07	0.87	0.91	1.62	1.36	1.45	P > P+NH > P+NO > C > NH=NO
	M	0.8	26.3	1.07	0.70	0.92	2.11	1.90	1.95	P > P+NH=P+NO > C > NH > NO
	D	1.8	27.0	1.06	0.82	1.16	3.11	2.63	2.82	P > P+NH=P+NO > NH > C > NO
	P	0.6	26.7	1.11	1.03	1.08	1.61	1.50	1.55	P=P+NH=P+NO > C > NH=NO

PRE = Premonsoon, MON = Monsoon, POS = Postmonsoon; H = Headwater, M = Midlake, D = Downlake Zone, P = Point-Source; Z = incubation depth, $t(°C)$ = water temperature, and C = control.

[1]Algal response in the controls and treatments are expressed as a $CHL_f : CHL_i$ ratios.

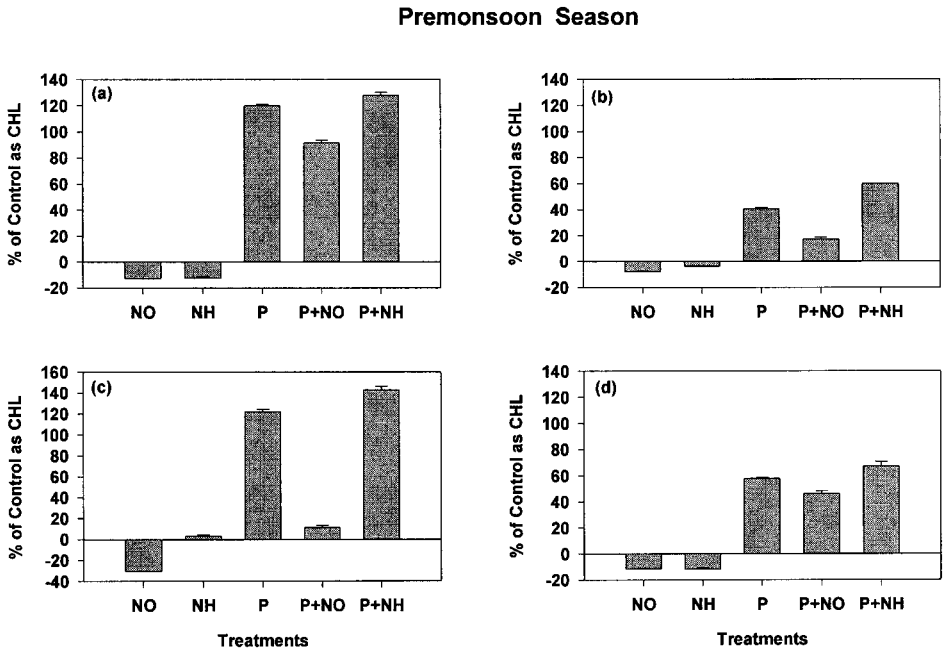

Figure 2. Spatial variation of Nutrient Enrichment Bioassays (NEBs) at the headwater (a), mid-lake (b), downlake (c), and point-source zone (d) during the premonsoon season. Algal response in the y-axis represent a chlorophyll (CHL) as % of the control [i.e., 100 * (treatment CHL–control CHL / control CHL)]. Error bar denotes one standard deviation. Treatments of NO, NH, P, P + NO, and P + NH indicate an enrichment of NO3-N, NH4-N, P, P + NO_3-N, and P + NH_4-N, respectively.

The response was least at the point-source site (Fig. 2); this reduced response may be due to continuous P-input (TP range: 45–57 μg L^{-1}) from the wastewater disposal plant or fish farms located nearby. Elser et al. (1993) pointed out that alteration of environmental conditions, especially incubating phytoplankton at favorable light intensities, may artificially increase the magnitude of algal response to nutrient limitation.[20] A variation in light condition, however, was minor during the incubation period; a photic depth, estimated as 2.3 * Secchi depth, was > 4 m at all sites and non-volatile suspended solids varied little (0.8–4.5 mg L^{-1}) among sites. In the mean time, the response in P + NO_3 treatments at all sites was significantly ($p < 0.05$) less than in P or P + NH_4-N treatments. In contrast, addition of P + NH_4-N, at all sites except the headwaters, showed a synergistic effect compared to P addition alone (Table 2) as shown in the nutrient bioassay of other lakes.[3] Addition of NH_4-N alone, however, yielded no greater growth than controls.

During monsoon season, P treatments at all sites showed significantly ($p < 0.05$) greater algal response than P + NH_4-N or P + NO_3-N treatments (Table 2). Response in the NO_3-N treatment was significantly ($p < 0.05$) less than the control. Phosphorus limitation was evident, but the magnitude of nutrient

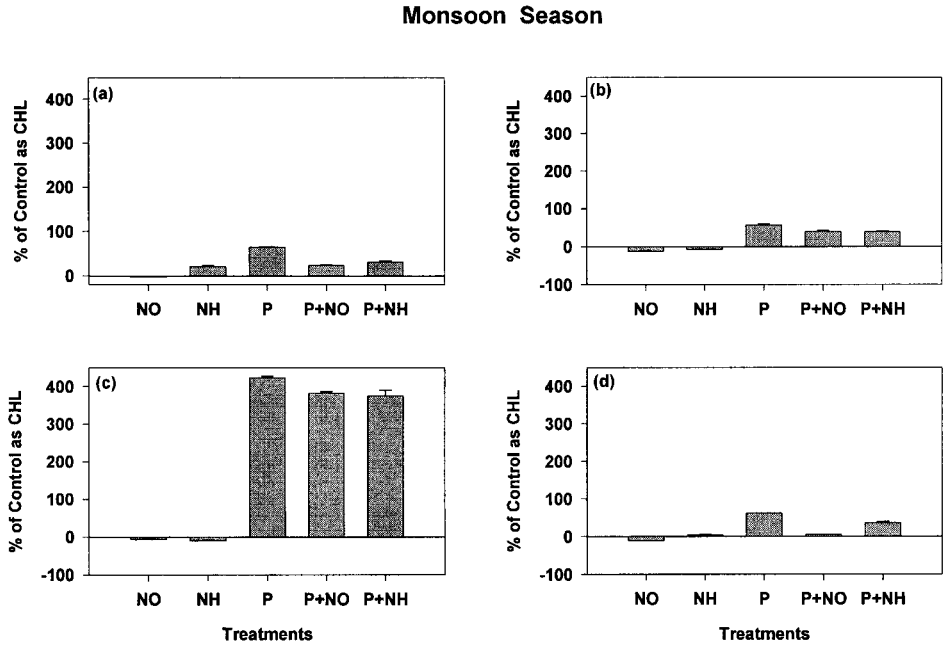

Figure 3. Spatial variation of Nutrient Enrichment Bioassays (NEBs) at the headwater (a), mid-lake (b), downlake (c), and point-source zone (d) during the monsoon season. Ditto with Fig. 1.

limitation varied among locations. The response in treatments enriched with phosphorus (P, $P + NH_4$-N, and $P + NO_3$-N) was greater downlake than elsewhere (Fig. 3C). Percent increases of CHL relative to control were >350% in the treatments downlake (Fig. 3C). In contrast, increases of CHL in the treatments at other sites were <90% of control (Fig. 3A, B, D). The greatest response downlake occurred during strong stratification in July when TP and NO_3-N in ambient water were minimal ($6\,\mu g\,L^{-1}$ and $0.87\,mg\,L^{-1}$, respectively) during the study (Table 1).

Treatments enriched with P (P, $P + NH_4$, and $P + NO_3$) during postmonsoon season were significantly ($p < 0.05$) greater than any other single or combination treatment (Table 2). Treatments enriched with P showed maximum algal growth downlake (Fig. 4C) and agreed with experiments during monsoon. Response in the nitrate treatments at all sites was significantly ($p < 0.05$) less than the controls, despite the fact that ambient levels of nitrate-N in the headwater, middle, and point-source sites had decreased about 2–5 fold relative to monsoon (July, Table 1). Increases of CHL relative to the control were <50% in all treatments at the headwater and point-source sites (Fig. 4). The reduced algal response at both sites is associated with decreases (<30) in the ambient N:P ratios, compared to the premonsoon and monsoon season (Table 1). Short-term NEBs in this system, based on three seasons, support the findings that the primary nutrient regulating algal growth.[4,5,7,8]

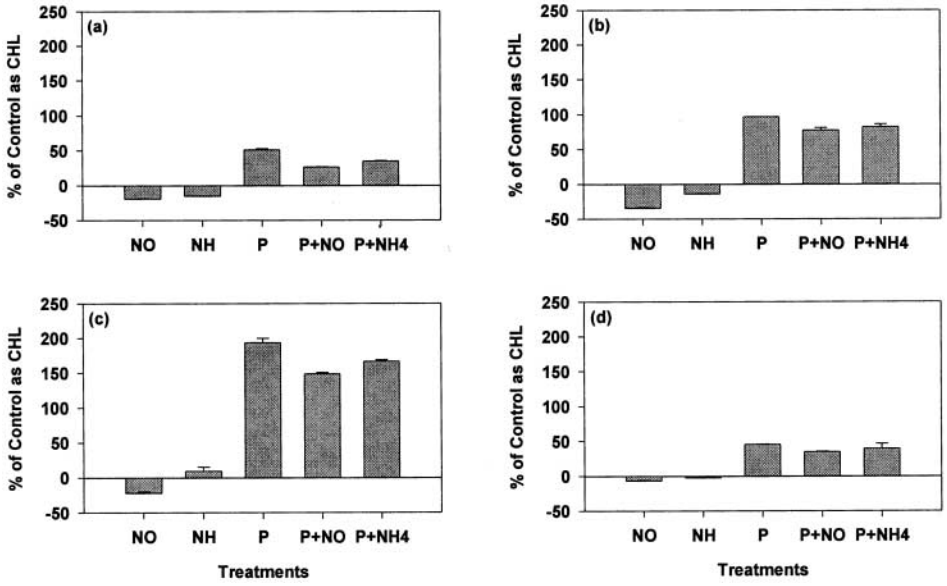

Figure 4. Spatial variation of Nutrient Enrichment Bioassays (NEBs) at the headwater (a), mid-lake (b), downlake (c), and point-source zone (d) during the postmonsoon season. Ditto with Fig. 1.

Seasonal Variation of NEBs

In situ NEBs showed that phosphorus limitation consistently occurred during all seasons (pre-, mon-, and postmonsoon), but the magnitude of P-limitation varied among seasons (Fig. 5).

All treatments enriched with phosphorus at the headwater site showed a significantly ($p < 0.05$) greater algal response than the NO_3-N or NH_4-N treatments (Table 2), indicating consistent P-limitation. P treatments during premonsoon stimulated growth by >80% relative to controls and showed a significantly ($p < 0.05$) greater response relative to experiments during the monsoon and postmonsoon (Fig. 5A). The greater response during premonsoon (9 June) relative to the monsoon and postmonsoon occurred under lowest ambient TP and highest TN:TP ratios at the headwater site (Table 1). This result may be explained by the inverse relation between in situ algal response and ambient TP level. Also, light availability (Secchi depth >2 m) might have contributed the algal response (Table 2). Percent CHL over the control in P treatments declined from premonsoon to monsoon (Fig. 5A). The seasonal decrease in algal response coincided with the progressive decline in N : P ratios.

Treatments enriched with phosphorus at the point-source site consistently showed a greater algal response relative to other treatments. However, the magnitude of response in treatments enriched with phosphorus varied from 50% to 70% over the controls among seasons (Fig. 5B), indicating low seasonal variation

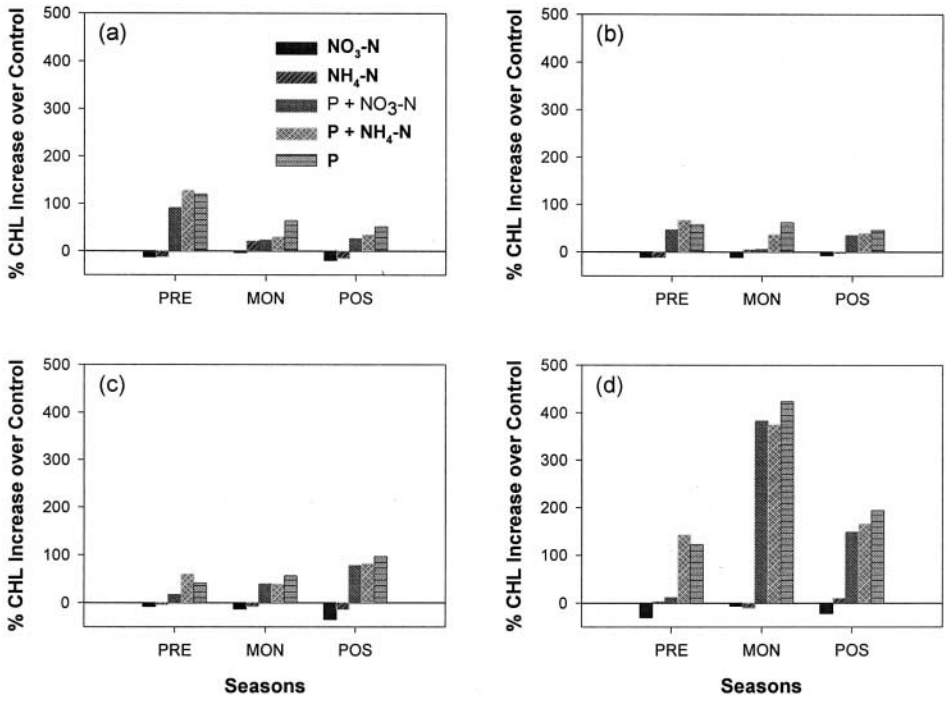

Figure 5. Temporal variation of NEBs at the headwater (a), point-source (b), mid-lake (c), and downlake zones (d). One control and five treatments were incubated at 1/2 Secchi depth and each treatment spiked 1/2 ambient concentration of nutrients.

compared to other sites. Algal growth in the NO_3-N treatment was significantly ($p < 0.05$) less than the controls, and this trend was consistent among seasons (Table 2). This reduced algal response may be because of low P variation (TP range: 48–57 $\mu g\, L^{-1}$) resulting from consistent nutrient loads from the point sources. In the mean time, at the middle site algal response in treatments enriched with phosphorus was greatest during postmonsoon among seasons (Fig. 5C). The response in the NO_3-N or NH_4-N treatments was least among treatments and did not vary among seasons.

At the downlake site, algal growth in NO_3-N or NH_4-N treatments was significantly ($p < 0.05$) less than the other treatments, and P-limitation occurred without regard to seasons (Fig. 5D). The yields of CHL in the treatments with added phosphorus was greatest during monsoon (Fig. 5D), resulting in algal growth of >370% over control. It is evident that P-limitation downlake was most severe during summer monsoon. This situation may be a result of a reduction in P by algal uptake and sedimentation of P during strong stratification with a decrease of external P by low inflow. This result supports the fact that variation in nutrient availability is a function of both external and internal processes.[20]

Algal Growth Rate Experiment

As shown in Table 3, algae usually responded within 3 days to P additions and the growth response was maintained for at least 9 days after enrichment. Within the first 3 days after enrichment, growth rates in treatments with phosphorus were significantly ($p < 0.05$) greater than the control or nitrogen treatments (NH_3-N and NO_3-N), and treatments enriched with nitrate-N showed slower growth rate than the control (Table 3). Ammonia addition with P showed significantly faster growth rate than the P addition alone, although the effect of NH_4-N treatment was similar to NO_3-N treatment alone, indicating a synergistic effect of NH_4-N with P. Growth rate in 1P and 2P treatments (1.28 and 1.63 $\mu g\,L^{-1}$ per day, respectively) was significantly ($p < 0.05$) greater than that in the 4P treatment (1.03 $\mu g\,L^{-1}$ per day). This phenomenon may be a result of growth retardation during the early incubation by an excessive P addition greater than required for growth (i.e., four times P addition of ambient lake-water) as shown in researches of other lakes.[21,22]

At 6 days after enrichment, growth rate in all P treatments was significantly ($p < 0.05$) greater than that in the control, NH_4-N, and NO_3-N treatments (Table 3). Compared to the rate on day 3, the growth rate in treatments of 1P, $P + NH_4$, 2P declined >40%, but the rate in 4P treatment (2.33 $\mu g\,L^{-1}$ per day) increased >100%. 4P treatment showed significantly ($p < 0.05$) higher growth rate than in 1P and 2P treatments (0.68, 0.93 $\mu g/L$ per d, Table 3). Also, the yield of biomass in 4P treatment was 3.8 times that of the control, and was >2 fold larger than 1P, 2P, $P + NH_4$-N and $P + NO_3$-N treatments, resulting a maximum growth rate (= 2.33 $\mu g\,L^{-1}$ per day) during the experiments.

Table 3. Algal growth rate experiment, based on the Nutrient Enrichment Bioassays (NEBs)[1].

D_s	IP	Z_m	Treatments (Ratios of $CHL_f : CHL_i$)							
30 May			C	NO	NH	P	P+NO	P+NH	2P	4P
	3	3.4	0.98	0.69	1.02	2.19	1.06	2.39	2.54	1.97
			ANOVA: 2P > P+NH > P > 4P > P + NO > NH > C > NO							
	6	3.4	1.39	1.02	0.97	2.28	1.89	2.41	2.76	5.38
			ANOVA: 4P > 2P > P + NH > P + NO > C > NO = NH							
	9	3.4	1.39	1.09	1.27	2.83	1.95	2.66	3.92	6.47
			ANOVA: 4P > 2P > P > P + NH > P + NO > NH = C > NO							
	12	3.4	1.45	1.27	1.13	2.19	2.03	2.78	4.47	6.94
			ANOVA: 4P > 2P > P + NH > P > P + NO > C > NH = NO							
	15	3.4	1.48	1.31	1.59	2.50	3.10	2.99	3.82	4.43
			ANOVA: 4P > 2P > P + NH = P+NO > P > C = NH = NO							

[1]Starting date (D_s) for the NEBs was 30 May and the serial incubation period (IP) was as follows: The incubation depth (Z_m, meter) was a half Secchi depth and the incubation temperature ranged between 20 and 23°C. Analysis of variance (ANOVA) test showed significant differences ($p < 0.05$) among treatment means at the same depth as determined by Duncan's Multiple Range test.

Nine days after enrichment, growth rate in 4P treatment showed a distinct decline, despite total biomass in the 4P treatment increased 20% relative to that on day 6 (Table 3). Also, growth rate in treatments enriched with 1P, 2P, $P + NH_4$ did not increase any more after day 9. Therefore, phytoplankton in cubitainers in this period was probably at or near the limit of available nutrients. In the mean time, twelve days after enrichment, algal biomass in 2P and 4P treatments showed a maximum yield by factors of 5 and 7, respectively, and decreased thereafter. The growth rate in the 2P and 4P treatments, however, declined from the previous sampling date (day 9).

In the overall growth experiment, growth rate in treatments enriched with NH_3-N, NO_3-N, and $P + NO_3$-N never exceeded $> 0.50\,\mu g\,L^{-1}$ per day and was significantly ($p < 0.05$) less than that in the three treatments with P (1P, 2P, and $P + NH_4$-N). In contrast, treatments with phosphorus, except for $P + NO_3$-N, showed a maximum growth rate of $> 1.20\,\mu g\,L^{-1}$ per day on day 3 or 6 in respond to added phosphorus (Table 3). It is evident that P was the primary nutrient regulating algal growth in this reservoir during this period as shown in lakes of North America and Europe.[4,5,7,8] Growth rate in the NO_3-N treatment, however, was always less than or same as the control. Early in the incubation period (3 d), the rate in treatments $P + NO_3$-N was significantly ($p < 0.05$) less than treatments with P, $P + NH_3$-N, and 2P. The reduced response in the treatments enriched with NO_3-N is unique because nutrient additions in general produce at least positive or no response in the bioassay experiments.

The suppression of nitrate on algal growth may be involved with bluegreen dominance of *Anabaena, Microcystis, and Oscillatoria* during the NEBs conducted in June–September. Previous studies demonstrated that high NO_3-N can suppress bluegreen growth and its addition can cause a compositional shift from bluegreen to green algae in whole-lake enrichment experiments. Hyensterand (1994) and Blomqvist et al. (1994) showed that algal response in nitrate-N treatments was significantly ($p < 0.1$) less than the control in other bioassay experiments.[21,23] A mechanism of the suppression may be explained by nitrate assimilation efficiency in bluegreens. Experimental approaches in chemostats[23–25] have demonstrated that bluegreens prefer ammonium over nitrate as a nitrogen source and have low-nitrate uptake rate in the high nitrate-rich environment. Under such circumstances, nitrogenase activity of bluegreens is inhibited,[26,27] resulting in a decrease in algal growth. In bioassay experiments, Klemer (1976) also found that the volume of *Oscillatoria* greatly increased in the treatment with $P + NH_4$, whereas it declined sharply in the treatment with $P + NO_3$.[28] A comparable result was found in these experiments of NEBs. These suggest a potential depression of phytoplankton growth under increased nitrate levels.

Additions of potassium in the NEBs, however, may have acted as another variable influencing algal growth. Investigators in short-term bioassay experiments[29–31] found that additions of potassium produced significant chlorophyll decreases. In the NEBs, this possibility may be because potassium in the cubitainers increased by 40 fold from both spikes of nitrate (KNO_3) and phosphorus (K_2HPO_4). Thus, excessive amounts of potassium added from both N and P spikes may have had a negative effect on algal growth or CHL synthesis. To elucidate these effects of potassium on algal growth, additional study should be done in the future.

Relations of Algal Response in the P treatments to Ambient Nutrients

Regression analysis of $\log_{10}$-transformed ratios of CHL_f to CHL_i against ambient nutrient contents showed that in situ algal response in the P treatments was inversely correlated ($r = -0.77$; $p < 0.001$) with ambient TP concentrations (Fig. 6). The magnitude of response increased longitudinally from the headwaters to downlake, and differences among the zones were significant ($p < 0.05$). This relation demonstrates that as TP values decreased downlake, the in situ algal response increased. These experiments support a general model of nutrient limitation

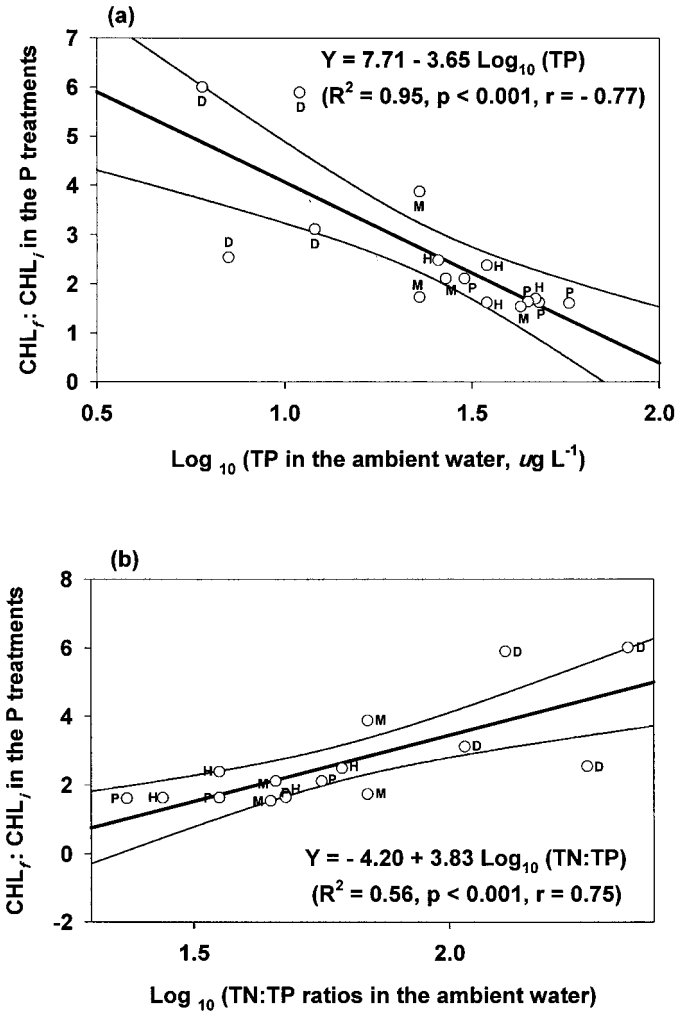

Figure 6. Regression analysis of algal response, expressed as ratios of CHL_f to CHL_i in the P treatments, against log10-transformed ambient TP (a) and ambient TN:TP ratios (b). Characters of "H", "M", "D", and "P" indicate the headwater, mid-lake, downlake, and point-source zone, respectively.

in reservoirs[11,32,33] in which nutrient availability decreases from uplake to downlake. Also, maximum response in P treatments in the downlake reach during summer monsoon supports the contemporary paradigm for temperate lakes that phytoplankton becomes increasingly limited by nutrients as the physical habitat progresses from cool and well mixed to warm and stratified.[34–35] This outcome in the NEBs is consistent the hypothesis that magnitude of algal response varies with spatial and temporal distribution of P in ambient water.[9,11] In most NEBs, strong algal response to the addition of P was attributed to low external P-input during the study period. This result agrees with the findings that limitation of phytoplankton abundance and growth depends largely on external loading of limiting nutrients of N or P.[9, 10] Also, as shown in Fig. 6, algal response was positively correlated with the $\log_{10}$-transformed TN : TP ratios ($r = 0.75$; $p < 0.001$). These relationships support the idea that algal response in P-limited systems is greater at the high N : P and the nutrient ratios is important factor regulating the phytoplankton growth.[9,11]

CONCLUSIONS

Nutrient contents and nutrient ratios in the ambient water indicated a potential phosphorus limitation for phytoplankton growth. In situ experiments of NEBs supported the evidence. At all sites, algal response in the P treatments was significantly greater than the control or nitrogen treatments, and response in the control was greater than nitrogen treatments. Among experiments conducted during June–September, phosphorus limitation was consistent regardless of the location, but the magnitude of growth response to P varied spatially and seasonally. The greatest response, in the spatial and temporal view, occurred in the downlake zone and during strong stratification of the monsoon, respectively. In the mean time, algal growth rate experiments in treatments enriched with nitrate-N showed an inhibition effect of nitrate on phytoplankton growth. This phenomenon may be explained by inhibition effect of nitrogenase activity through low-nitrate uptake rate in the high nitrate-rich environment and large additions of potassium with phosphorus in spike. Regression analysis of CHL_f : CHL_i ratios against ambient phosphorus suggested that as phosphorus concentrations decreased downlake, the in situ algal response increased. These outcomes provide an efficient management strategy for the lake water quality control.

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