Original Research

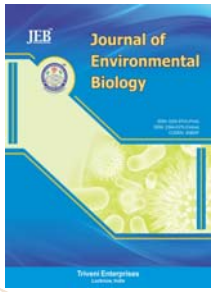
Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/38/4/PRN-103



In situ nutrient-spiking bioassays for determining phosphorus and light limitation in a wetland ecosystem

Authors Info

Woon-Ki Moon, Ji-Woong Choi, Sang-Jae Lee and Kwang-Guk An*

Department of Biological Sciences, College of Biological Sciences and Biotechnology, Chungnam National University, Daejeon - 305 764, Korea

*Corresponding Author Email : kgan@cnu.ac.kr

Key words

Algal growth,
Nutrient spiking bioassay,
Phosphorus-limitation,
Suspended solids,
Wetland ecosystem

Publication Info

Paper received : 23.11.2015
Revised received : 15.03.2016
Re-revised received : 10.10.2016
Accepted : 09.11.2016

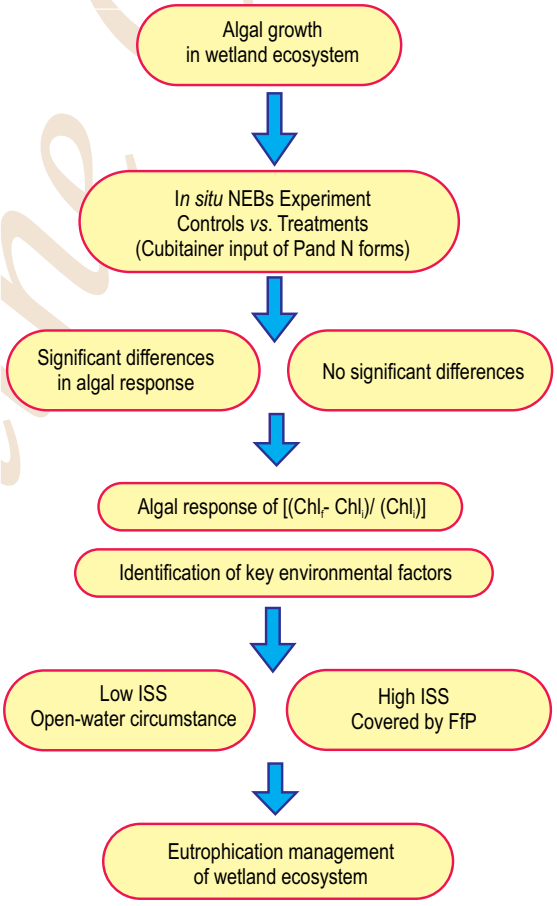
Abstract

Aim : The objective of the present study was to determine the factors that regulate the response of algal growth in wetland ecosystem by *in situ* nutrient-spiking bioassays (NSBs).

Methodology : The NSBs tested algal responses as a ratio of final (Chl_f) to initial Chl (Chl_i) in the control (no nutrient addition) and several treatments of phosphorus (P), nitrogen (NH_4-N, NO_3-N), and NO_3-N+P under various environmental conditions of inorganic suspended solids (ISS) and free-floating plants (F_fP).

Results : Experiments of *in situ* NSBs with low inorganic solids showed that the response of P treatments were significantly ($p< 0.05$) greater than the controls and the treatments of NH_4-N or NO_3-N. Regression analysis of Chl_f : Chl_i ratios against log10-transformed TP in the cubitainers, thus showed that *in situ* algal response in P treatments was directly determined ($R^2 = 0.965$, $F = 40.049$, $p< 0.001$) by concentrations of spiked TP. In contrast, the *in situ* NSBs with high ISS (> 15 mg l^{-1}) or high FfP cover (> 95%) showed that the response in treatments of N, P or P+NO_3-N had no significant ($p> 0.05$) difference with the controls.

Interpretation : Light limitation dominated the system, thus the ratio of Chl_f : Chli had no relation ($p> 0.10$) with the spiked TP. Overall, the experiments of *in situ* NSBs suggest that high P-inputs from the watershed increased algal growth, but dense F_fP or high inorganic solids suppressed eutrophication in the wetland.



Introduction

Wetland studies are recognized as one of hot ecological research topics for conservation of biodiversity and wildlife habitats. They have diverse hydrological and ecological functions such as flood control (Ogawa and Male, 1986; Hey and Philippi, 1995), providing natural habitat for aquatic biota (Ralph and Rogers, 2011), water purification (Gersberg *et al*., 1986), nutrient retention balancing terrestrial and aquatic ecosystems (Craft CB, 1997), sediment filtering capacity from the watersheds (Mc Jannet *et al*., 2012), as well as aesthetic and economic aspect of ecological tourism. For these reasons, water pollution and eutrophication are largely focused in the wetlands (Brix, 1994), and nutrients such as nitrogen and phosphorus have been primary chemicals regulating the wetland productivity (Elser *et al*., 2007), although chemicals such as carbon and silica (diatom) have frequently been proposed as important (Goldman *et al*.,1972; Struyf and Conley, 2008). Phosphorus in wetland ecosystems is lost from the water column by sedimentation processes and has no gas phase, so it is often considered the most important limiting nutrient, which thereby regulates eutrophication of wetlands (Reddy *et al*., 1995). The input-output models (Vollenweider, 1975, 1976) or empirical models (Mitsch and Reeder, 1991) show how nutrients and chlorophyll or primary production, are functionally related to external loading or input of nitrogen and phosphorus from the watersheds.

Many published studies on wetlands have pointed out that phytoplankton dominatein waterbodies with a large phosphorus input (Pan *et al*., 2000), low underwater light regimes (O'Farrell *et al*., 2007), high water temperature (Kadlec and Reddy, 2001), water column stability (Reynolds and Walsby, 1975) or low N:P ratio (Güsewell *et al*., 2003). Nitrogen and phosphorus have been considered as key variables for algal blooms and the magnitude of wetland eutrophication is closely related to supply ratios of these two key nutrients along with under water light availability. In particular, nutrien loading of P or N in temperate Asian monsoon regions is controlled by frequency and intensity of monsoon rainfall or runoff during the monsoon periods.

Lentic and lotic ecosystems in Asian regions potentially have unique trophic characteristics determined by the summer monsoon. One third or half of total annual precipitation in Korea occurs during short period summer monsoon July - August. Thus, nutrients, primary production, and light regime may largely vary by them on soon flood events, which will result in short hydraulic residence time (An and Park, 2002).Especially, large inputs of non-volatile suspended solids (NVSS) during flood can reduce the underwater light availability, and may reduce phytoplankton biomass relative to available nutrient supplies. This phenomenon is frequently observed in wetland ecosystems of Asianregions (Muzaffar and Ahmed, 2007). Under these circumstances, phytoplankton production orgrowth may be closely associated light availability rather than by amounts of nutrients, and phytoplankton growth is largely reduced. Empirical relation between nutrients (N, P) and chlorophyll-a or between chlorophyll-a and nutrient regime, thus, may be modified, resulting inmodification of ecological functions in the wetland ecosystems.

Assessment of trophic variables such as nitrogen, phosphorus, or chlorophyll, are important in the wetland ecosystems, but more important thing is to evaluate the chlorophyll-a response to the trophic variables using an in situ cubitainer approach like nutrient-spiking bioassay. The technique of nutrient enrichment bioassays or nutrient-spiking bioassays (NSBs) is easy to conduct in fields,and iscost effective in diagnosing the nutrient response to phytoplankton growth and are widely applied for determining nutrient or light limitations in wetl and,lake and stream ecosystems (Elser *et al.,* 2007; Pulatsu and Topcu, 2009). Various nutrient-spiking bioassays such as short-term batch culture method, radioactive nutrient or whole lake approaches employing in situ polyethylene cubitainers are used and especially,in situ bioassays using enclosures or cubitainers have been commonly used for identification of key or primary nutrients most limiting in aquatic ecosystems (Elser *et al.,* 1990).

The aim of the research was to apply a new approach of *in situ* nutrient stimulation experiments (NSEs) for determining phosphorus and light limitation in Woopo Wetland, and evaluate temporal variability of algal growth responses under different wetland conditions such as dominance of non-volatile suspended solids (NVSS) or free-floating plants (F_fP) in wetland ecosystem.

Materials and Methods

Experimental designs of nutrient-spiking bioassays : *In situ* nutrient stimulation experiments (NSEs) were conducted in Woopo Wetland, which is located in the southern part of South Korea and influenced by Asian monsoon. For the experiments, two different sites (W1, W2) with a dominance of non-volatile suspended solids and free-floating plants (F_fP; *i.e*., *Spirodela polyrhiza*), respectively were selected to determine nutrient or light limitations of algae. Surface water was mixed in a 120-L polyethylene-lined container and then dispensed into 10-L translucent polyethylene cubitainers (Nalgene Company). Unfiltered surface waters were suspended at 0.5m depths and incubated 3 days in the cubitainers. Five different individual bioassays (BIO-I to BIO-V) had controls and various treatments consisting of additions of N and/or P, with two or three replicates each.

Temporal effects of bioassays in relation to water temperature, free-floating plants (F_fP), and inorganic solids were tested in each bioassay. First tests of BIO-I were conducted during summer season (July-August), with intense rainfall and runoffs from the watershed, and inorganic suspended solids (ISS) dominated in the water-column of wetland along with high inorganic turbidity (> 70 NTU). The bioassays of BIO-II were conducted in the fall season (November-December) with 10 - 15

℃ water temperature and reduced aquatic floating plants, and the BIO-III was conducted during freezing winter season (January-February). The bioassays of BIO-IV and BIO-V were conducted in the growing season (May-June) with hydrologically stable water-column and hot summer season, respectively.

***In situ* nutrient-spiking and experimental conditions :** The first bioassays (BIO-I) were conducted to determine which nutrient (N or P) is a primary element regulating the algal productivity in this system. The experimental design included one control (C, no nutrient addition), and four treatments of nitrate-nitrogen (1.11 mg l^{-1} as NO_3-N), ammonia-nitrogen (0.40 mg l^{-1} as NH_4-N), phosphorus (105 µg l^{-1} as PO_4-P), and simultaneous addition of nitrate-nitrogen (1.11 mg l^{-1} as NO_3-N) plus phosphorus (105 µg l^{-1} as PO_4-P). The experiments of BIO-I was conducted under three different conditions as follows; 1) low inorganic suspended solids = ISS: < 5 mg l^{-1}) and low coverage of free-floating plants (F_fP; < 5%) on the surface water, 2) low ISS and high F_fP dominance (90 - 95%) in the surface, and 3) moderate ISS (range: > 15 mg l^{-1}) and low F_fP coverages (< 5%).

The experiments of BIO-II tests were conducted to determine algal response under the condition of low or absence of free-floating plants (F_fP) with no influence of inorganic suspended solids. The 2nd bioassays of BIO-II included the followings: control (C) and four treatments of nitrate-nitrogen (1.10 mg l^{-1} as NO_3-N), ammonia-nitrogen (0.70 mg l^{-1}), phosphorus (100 µg l^{-1} as PO_4-P), and simultaneous addition of nitrate-nitrogen (1.10 mg l^{-1} as NO_3-N) plus phosphorus (100 µg l^{-1} as PO_4-P). The bioassays of BIO-II were conducted under two different conditions as follows; 1) low ISS (range= 3.1 - 6.3 mg l^{-1}, mean = 4.9 mg l^{-1}) and absence of F_fP, and 2) low ISS (mean = 2.3 mg l^{-1}) and partial coverage of F_fP (15-20%) in the surface.

The tests of BIO-III had controls (C) and four treatments of nitrate-nitrogen (0.80 mg l^{-1} as NO_3-N), ammonia-nitrogen (0.30 mg l^{-1}), phosphorus (50 µg L^{-1} as PO_4-P), and simultaneous addition of NO_3-N (0.8 mg l^{-1}) plus PO_4-P (50 µg l^{-1}). The tests of BIO-III were conducted at the winter season (mean water temperature of 3.3℃) and specific conductivity of 428 µ*S* cm^{-1}. Also, the water had a condition of low ISS (< 2 mg l^{-1}) and no free-floating plants (F_fP) on the surface water. *In situ* tests of BIO-IV had controls (C) and four treatments of NO_3-N (1.20 mg l^{-1}), NH_4-N (0.60 mg l^{-1}), PO_4-P (50 µg l^{-1}), and simultaneous addition of NO_3-N (1.20 mg l^{-1}) plus PO_4-P (50 µg l^{-1}). The tests of BIO-IV were followed by the approach of BIO-III. During the bioassays of BIO-IV, F_fP started to grow, thus the surface water was rarely covered by floating plants. In the meantime, the tests of BIO-V had controls (C) and four treatments of NO_3-N (1.20 mg l^{-1}), NH_4-N (0.80 mg l^{-1}), PO_4-P (100 µg l^{-1}), and simultaneous addition of NO_3-N (0.80 mg l^{-1}) plus PO_4-P (100 µg l^{-1}).

In the spiking bioassays, ammonium nitrate (NH_4NO_3) was used as the source of respectively nitrogen, and potassium phosphate (K_2HPO_4) was used in the phosphorus treatment. Cubitainers of 10-L in the total volume for the bioassays (I - V) were suspended during 3 - 5 days at the subsurface of 0.6 m. In these experiments of nutrient-spiking bioassays, the response of phytoplankton to the controls and treatments was determined by measuring chlorophyll-a (Chl) in the bioassay cubitainers. The response in the bioassay was expressed as the ratio of final (CHL_f) to initial Chl (Chl_i) or ratios of final Chl in each treatment (T_{CHL}) to Chl in the control (C_{CHL}). Statistical differences among the controls and treatments were analyzed by one-way ANOVA Duncan's multiple range test.

Results and Discussion

The nutrient-spiking bioassays (NSBs) in summer season conducted to test the effects of floods on the nutrient or light limitation showed that algal productivity response, based on the ratios of (CHL_f - CHL_i)/CHL_i, varied largely depending on the environmental conditions of wetland (Fig. 1). Under low coverage of free-floating plants (F_fP; < 5%) with low ISS (< 5 mg l^{-1}), algal response coefficient in the treatments of P and NO_3-N+P was 0.73 and 0.65, respectively, while the coefficient in the controls and NO_3-N treatments was 0.13 and 0.11 (Fig. 1a). The treatments of NO_3-N+P enriched with nitrate-N plus phosphate-P, thus, were significantly (p< 0.05, ANOVA tests) greater than the control (C) or treatments enriched with nitrate-nitrogen (Fig. 1a). The productivity response in the control did not differ from treatments of NO_3-N. This suggests that primary element regulating the algal productivity in this system is phosphorus and nitrate-N did not influence the algal growth. This outcome differs from the finding (Vincent *et al*., 1984) that nitrogen limitation was frequently observed in the Asian regions (Shardendu, 2009). Under this situation, phosphorus input from the watershed would result in rapid eutrophication and increase of algal growth rate.

Under the high coverage of free-floating plants (F_fP; 90 - 95%) on the surface with low ISS (~ 3.4 mg l^{-1}), the response coefficients in the control and all treatments were < 0.1 (Fig. 1b), indicating no responses to the nutrient additions of N and P. Thus, any nutrient limitations on the algal growth did not occur under the circumstance of low ISS and high dominance of F_fP in the surface (Fig. 1b). Both treatments of P and NO_3-N+P did not differ from the controls or any other nitrogen treatments of NH_4-N and NO_3-N, indicating that P-limitation did not occur in this bioassay. In contrast, final algal productivity in the treatments of NH_4-N and NO_3-N decreased compared to the initial values, even if no statistical significance occurred between the two periods (Fig. 1b). The outcome of bioassay implies that only light limitation occurred without any nutrient limitation (N, P).

In the bioassays of moderate ISS (> 15 mg l^{-1}) and low coverage of free-floating plants (F_fP; < 5%), algal response coefficient in the treatments of P and NO_3-N+P was 0.27 and 0.29, respectively, while the coefficient in the controls and NO_3-N+P treatments was 0.06 and 0.08 (Fig. 1c). Both treatments of P and NO_3-N+P, thus, were significantly (p< 0.05) greater than the control (C) or treatments enriched with nitrate-nitrogen and the

response in the controls (C) did not differ from the NO_3-N treatment (Fig. 1c). These conditions indicate that a partial light limitation was due to decreases of underwater light by inorganic solids (non-volatile suspended solids) in the bioassay.

In situ nutrient-spiking bioassays (NSBs) during fall season (November - early December) showed that the ratio of $(Chl_f - Chl_i)/Chl_i$, in the treatments of P and NO_3-N+P was 0.66 and 0.59, respectively and the ratios in the control, NH_4-N and NO_3-N treatments were < 0.25. Two treatments of P and NO_3-N+P were significantly (p< 0.05, ANOVA tests) greater than the control (C) or treatments enriched with nitrate-nitrogen or ammonia-nitrogen (Fig. 2a). This was due to increased light availability by low ISS (range= 3.1 - 6.3 mg l^{-1}, mean = 4.9 mg l^{-1}) and the absence of free-floating plants (F_fP; < 5%) in the surface, even if the mean water temperature were low (9.2 - 12.2℃) compared to BIO-I. $(Chl_f - Chl_i)/Chl_i$, in the treatments of P and NO_3-N+P of Exp-II was 0.54 and 0.49, respectively, which were significantly (p< 0.05) greater than the control (C) or the treatments enriched with nitrate-nitrogen or ammonia-nitrogen (Fig. 2b). The ratios in the treatments of P and NO_3-N+P were decreased in the Exp-I than in the Exp-II, and this was mainly attributed to a reduced underwater light of partial coverage of F_fP (< 5%). In other words, P-limitation was consistent in the postmonsoon period.

The nutrient-spiking bioassays (NSBs) tests during the winter season showed that two treatments of P and NO_3-N+P were significantly (p< 0.05, ANOVA tests) greater than the control (C) or treatments enriched with nitrate-nitrogen or ammonia-nitrogen (Fig. 2c) and the magnitude of productivity response to P addition was statistically greater than the ratios in the P-treatment of BIO-I and BIO-II (Fig. 1 and Fig 2). The greater responses in the NSBs were due to high water transparency (low ISS of < 2 mg l^{-1}) and no free-floating plants (F_fP), resulting in greater algal productivity. These outcome suggests that in the Asian monsoon regions phytoplankton growth is greater in the winter than summer season. Our seasonal pattern in the nutrient stimulation bioassays is opposite to those in the water bodies of North America and Europe where the algal response is greater in summer than in the winter. Our reduced algal response during summer season in the NSBs was a results of largely increased inorganic turbidity (high NVSS) and high coverage of F_fP, in spite of increased phosphorus in the ambient water. Also, up-stream monsoon runoff during the summer increased P-input in the system and this P was mainly composed of particulate phosphorus (PP), which is not available for the algal growth. In fact, mean ratio of PP:TP in the summer monsoon was 0.82, but was 0.11 in the winter season, resulting in decreases of particulate fractions of > 7-fold in the winter.

In situ tests during spring season also indicated a P-limitation on the algal productivity response and nitrogen treatments enriched with ammonia-N and nitrate-N did not differ from the control (C). The ratio of $(Chl_f - Chl_i)/Chl_i$, in the treatments of P and NO_3-N+P was maximized during the spring of growing season (Fig. 2d), thus the magnitude of productivity response to phosphorus addition was greatest. This was due to increased water temperatures (15.3 - 20.0°C) along with light availability by

Table 1 : *In situ* Nutrient Spiking Bioassays (NSBs), based on the ratios of Chl_f/Chl_i in the controls (C) and various treatments of ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), phosphorus (P), and nitrate-nitrogen + phosphorus (NO_3-N+P). Analysis of variance (ANOVA) tests were based on significant statistical differences of p < 0.05. The marks of † and * indicate the NSBs' of 2003 and 2002, respectively (T_a = all treatments)

Period	Control (C)	Ratios of Chl_f: Chl_i in the NSBs				
		NH_4-N treatment	NO_3-N treatment	P treatment	NO_3-N+P treatment	Mean response (T_a:C ratios)
Jul 03†	1.134	1.214	1.113	1.733	1.655	1.260
		ANOVA Tests: P = NO_3-N+P > NH_4-N > NO_3-N = C				
Jul 15†	0.906	0.765	0.848	1.054	1.053	1.026
		ANOVA Tests: P = NO_3-N+P > NH_4-N = NO_3-N = C				
Aug 03†	1.063	1.133	1.077	1.267	1.243	1.134
		ANOVA Tests: P = NO_3-N+P > NH_4-N > NO_3-N = C				
Nov 10†	1.026	1.092	1.017	1.658	1.593	1.306
		ANOVA Tests: P = NO_3-N+P > NH_4-N > NO_3-N = C				
Dec 03†	1.022	1.125	0.957	1.537	1.489	1.250
		ANOVA Tests: P = NO_3-N+P > NH_4-N > NO_3-N = C				
Feb 16 *	1.150	1.174	0.818	2.000	1.909	1.283
		ANOVA Tests: P = NO_3-N+P > NH_4-N = C > NO_3-N				
May 15 *	1.063	1.206	1.167	2.278	2.235	1.619
		ANOVA Tests: P = NO_3-N+P > NH_4-N > NO_3-N = C				
Sep 12 *	0.750	0.786	0.667	0.846	0.833	1.044
		ANOVA Tests: P = NO_3-N+P = NH_4-N = NO_3-N = C				

low ISS (range= 2.2 - 3.8 mg l^{-1}, mean = 2.8 mg l^{-1}) and the absence of free-floating plants (F_fP) in the surface. In contrast, the ratios of (Chl_f - Chl_i)/Chl_i in the control and all treatments were negative (range: -0.17 ~ -0.33; Fig. 2e) during the early summer when the flood did not occur and the F_fP covered the surface up to 100%. During the experiments of NSBs, mean ratio of DP:TP in the ambient water was 0.92 and mean concentration of NVSS was 2.8 mg l^{-1}, indicating a high availability of P and low inorganic turbidity. But, F_fP blocked the underwater penetration during this period. Thus, the responses of algal productivity to the nutrient additions of N or P did not occur in the early summer season. *In situ* tests during the early summer, suggest that light limitation was evident for the phytoplankton growth.

Our experiments NSBs indicated that *in situ* responses of NSBs, expressed as Chl_f:Chl_i, in the treatments of P were always greater than the values of NO_3-N+P treatments, except for the season covered completely by free-floating plants (F_fP), even though the ratios of P-treatment did not statistically (p< 0.05) differ from the NO_3-N+P treatments (Table 1). These results suggest that P limited the phytoplankton growth, but additions of nitrate-N suppressed the phytoplankton growth in this wetland ecosystem. The influences of nitrate-N spiking on the phytoplankton growth, however, were minor because of no differences (p< 0.05) between the controls and NO_3-N treatments. Such possibility of growth inhibitions by nitrate-N are supported by the findings of Blomqvist *et al.*, (1994) who reported that algal response in nitrate-N treatments was significantly (p < 0.1) less than the control in other nutrient enrichment bioassays. A mechanism of the suppression may be explained by nitrate assimilation efficiency in cyanobacteria, which dominated the phytoplankton community in the system. Experimental approaches in chemostats have demonstrated that this type of algae preferred ammonium over nitrate as a nitrogen source and had low-nitrate uptake rate in the high nitrate-rich environment (Blomqvist *et al.* 1994). In fact, concentrations of nitrate-N were > 1.10 mg l^{-1} in the ambient water of our wetland ecosystem regardless of the seasons and locations. Under this circumstance, therefore, nitrogenase activity of cyanobacteria might have inhibited the algal growth (Leonardson and Ripl 1980; Blomqvist *et al.* 1994), resulting in a decrease in algal growth. In the meantime, there were no significant differences (p< 0.05, ANOVA tests) among the controls and all treatments of P, NO_3-N, NO_3-N+P, and NH_4-N under the circumstance of 100% coverage of free-floating plants (F_fP) in the surface water. Elser *et al.* (1990) 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. A variation in light condition, however, was maximized during the incubation period; a photic depth was nearly zero due to the surface cover of F_fP, even if non-volatile suspended solids were low (mean = 2.4 mg l^{-1}; range: 1.8 - 2.9 mg l^{-1}) in the season. Also,

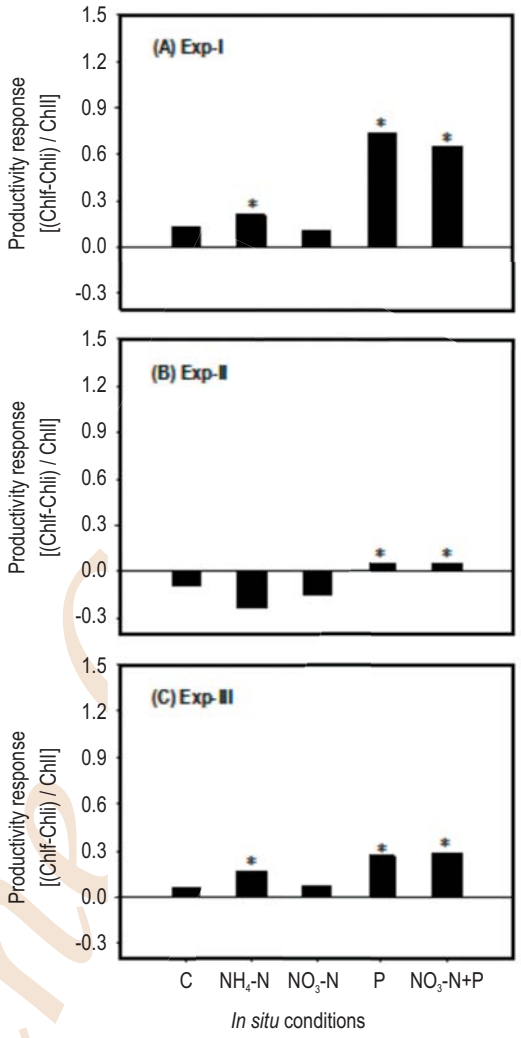


Fig. 1 : *In situ* flooding season experiments of nutrient-spiking bioassays (NSBs) under three different conditions of low inorganic suspended solids (ISS) of < 5 mg l^{-1} without free-floating plants (F_fP, Exp-I; a), massive dominance of F_fP with low ISS of < 5 mg l^{-1} (Exp-II; b), high ISS of > 15 mg l^{-1} without the F_fP (Exp-III; c). Algal productivity response was expressed as a ratio of (Chl_f - Chl_i)/Chl_i, in the nutrient spiking experiments (NSEs), which contained the control (C) and four treatments of NH_4-N, NO_3-N, P and NO_3-N+P

the treatments of NO_3-N showed significantly (p< 0.05) greater or same algal response than the NH_4-N treatments (Table 1), indicating that ammonia-nitrogen influence the algal growth more than the nitrate-N, even though P-limitation is evident. These outcomes imply that ammonia-N might have stimulated the algal response weakly.

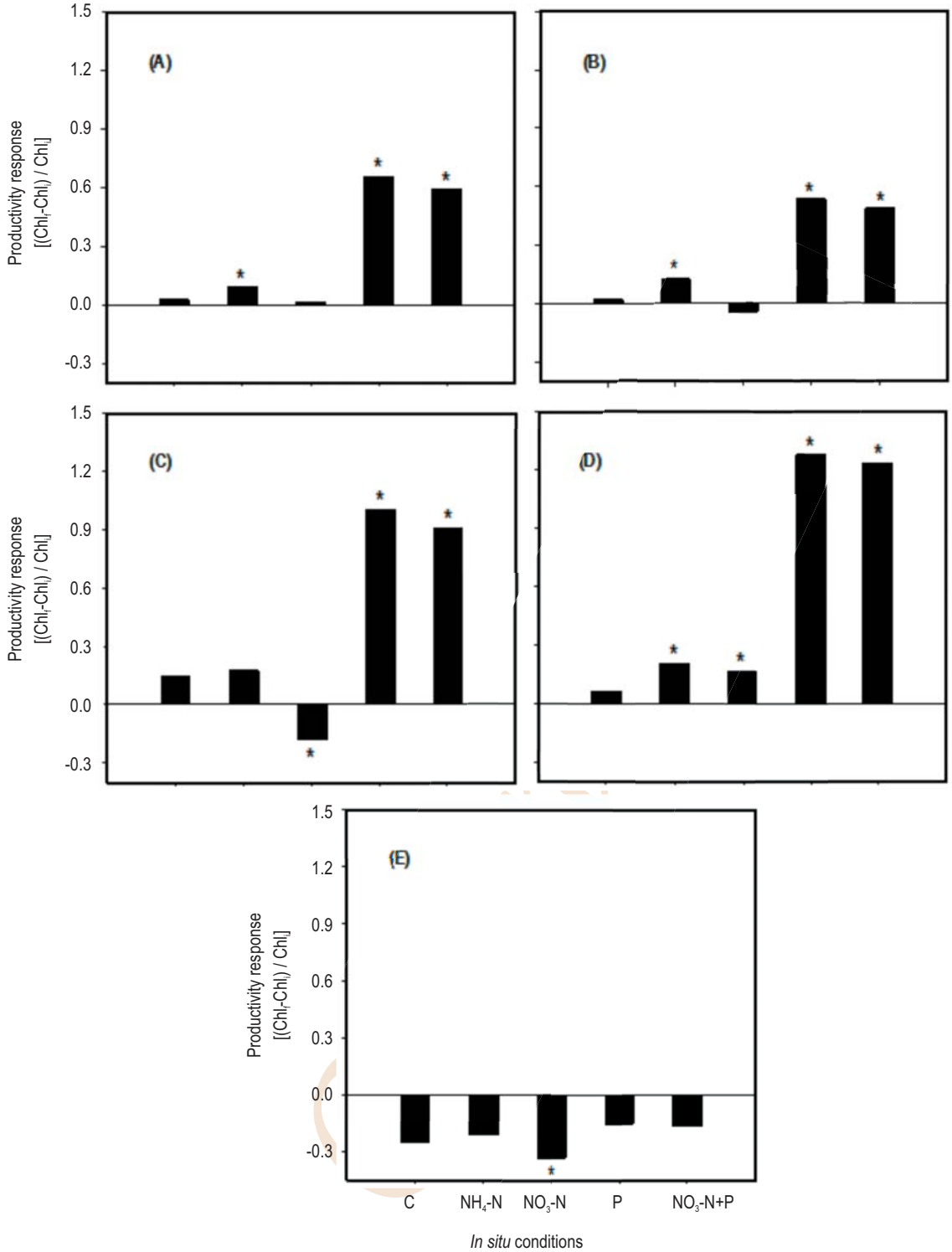


Fig. 2 : Algal productivity response as an equation of (Chl$_f$-Chl$_i$)/Chl$_i$in the nutrient-spiking experiments (NSEs). Experimental designs of NSEs are as follows; low ISS of < 5 mg l^{-1} without the free-floating plants (F$_f$P) at autumn (a), partial growth of F$_f$P with low ISS of < 5 mg l^{-1} at early winter (b), no F$_f$P on the surface water with low ISS of < 2 mg l^{-1} at late winter (c), growth starting of F$_f$P at early spring, (d) and 100% cover of the surface by F$_f$P with low ISS (< 3 mg l^{-1}) during summer (e)

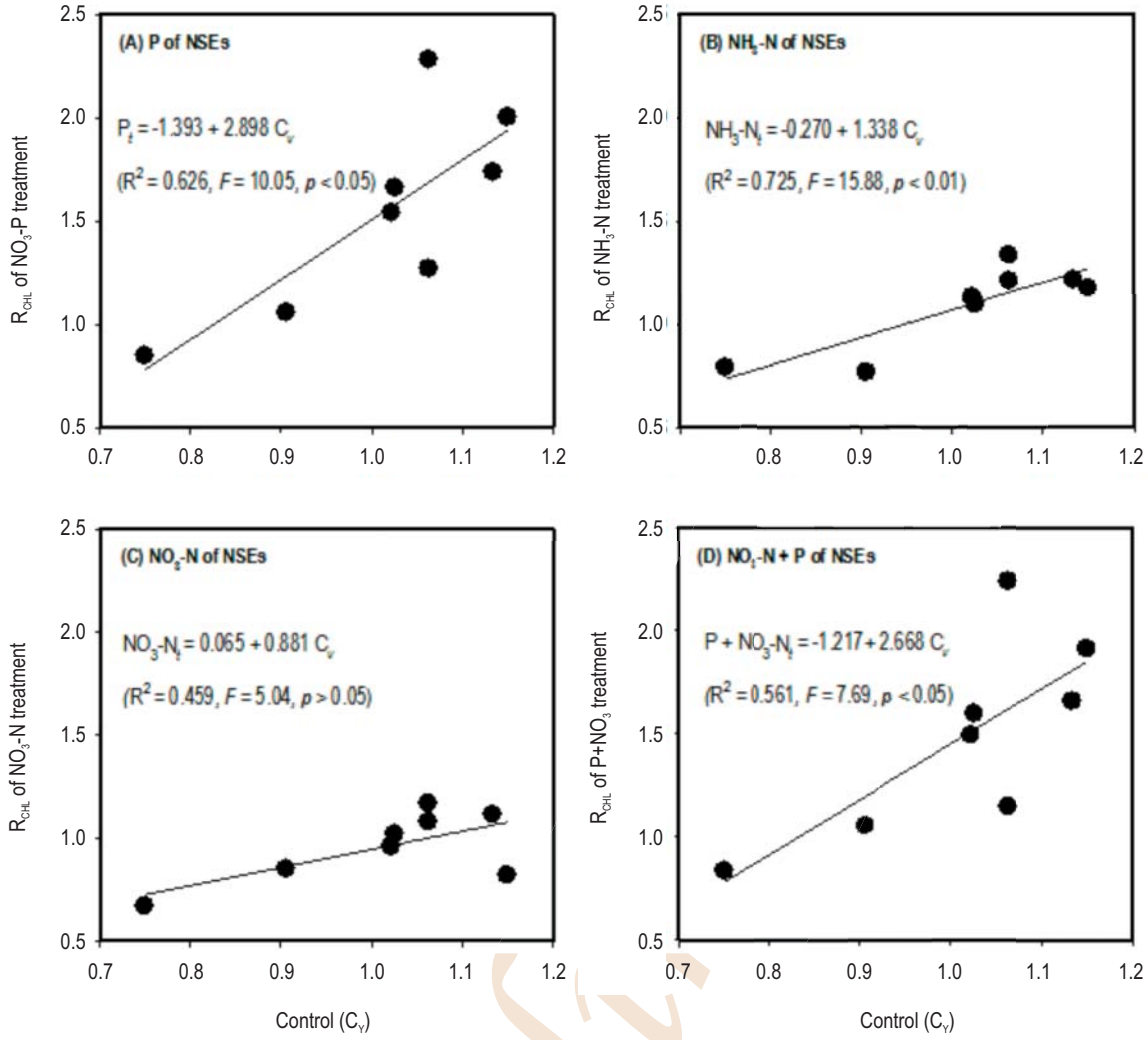


Fig. 3 : Linear regression analysis on the response of Chl (R_{CHL}) of phosphorus (P), ammonia-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and nitrate-nitrogen +phosphorus (NO_3-N+P) treatments against the controls (C_v) in the nutrient-spiking experiments (NSEs) of translucent cubitainers

The relation of nutrient-spiking to control (C_v) in the response of Chl (R_{CHL}) also supported the phosphorus-limitation in the system (Fig. 3). Treatments of P and NO_3-N+P in the NSEs showed significant increases of Chl (R^2 = 0.626, p< 0.05; R^2 = 0.561, p< 0.05) compared to the control, and the response of Chl in the P and NO_3-N+P treatments increased up to 2.27 and 2.23, respectively (Fig. 3). In contrast, there was no significant relations (p = 0.065) between R_{Chl} of NO_3-N and C_v, and the all response values of Chl in the NH_3-N was < 1.5 (Fig. 3), indicating that ammonia or nitrate-nitrogen addition did not increased the algal growth.

Overall, *in situ* experiments of no floods or no free-floating plants (F_fP) showed that in 5 of total 8 NSBs, additions of phosphorus alone or with nitrate-N caused a significant increase of algal growth relative to the controls (Fig. 4). The regression analysis of CHL_f:CHL_i ratios against Log_{10}-transformed ambient TP in the cubitainers showed that *in situ* algal response in P treatments was directly determined (R^2 = 0.965, F = 40.049, p< 0.001) by ambient TP (Fig. 4a) when the density of F_fP was low on the surface and non-volatile suspended solids were < 5.0 mg l^{-1}; Ratios of Chl_f : Chl_i = -3.1260 + 2.06 Log_{10} (TP). In contrast, the ratios of Chl_f : Chl_i had no relations with ambient TP in the

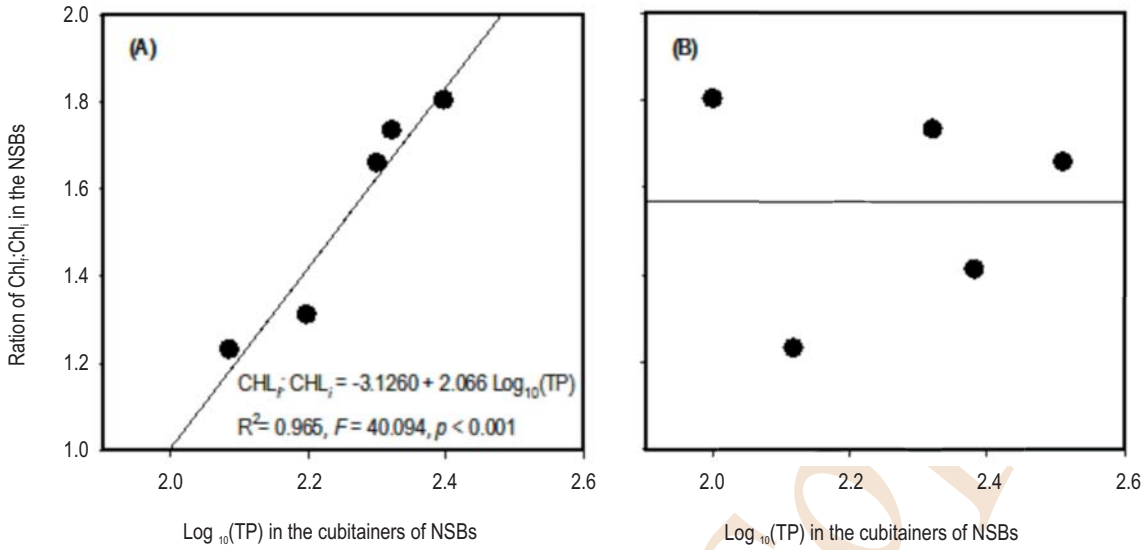


Fig. 4 : Ratios of final to initial chlorophyll (Chl$_f$: Chl$_i$) as a algal response on addition of phosphorus (P) (a) when the *In situ* bioassays were conducted at no floods or no free-floating plants (F_fP) and (b) when the F_fP covered the surface completely or mean NVSS was > 15.0 mg l^{-1}

cubitainers (Fig. 4b) when the system was completely covered by F_fP and non-volatile suspended solids were > 15.0 mg l^{-1}. Under the circumstances, no nutrient limitations of P or N occurred in this system and only light limitation was key factor regulating the phytoplankton growth.

Acknowledgments

This research was supported by Basic Science Research Program through the NRF of Korea (NRF) funded by the Ministry of Education (No. 2013R1A1A4A01012939) and Daejeon Green Environment Center under the Research Development Program (Yr 2009).

References

An, K.G. and S.S. Park: Indirect influence of the summer monsoon on chlorophyll–total phosphorus models in reservoirs: A case study. *Ecol. Model.*, **152**, 191-203 (2002).

Blomqvist, P., A. Pettersen and P. Hyenstrand : Ammonium nitrogen: A key regulatory factor causing dominance of non-nitrogen fixing cyanobacteria in aquatic systems. *Arch. Hydrobiol.*, **132**, 141-164 (1994).

Brix, H.: Use of constructed wetlands in water pollution control: Historical development, present status, and future perspectives. *Water. Sci. Technol.*, **30**, 209-224 (1994).

Craft, C.B.: Dynamics of nitrogen and phosphorus retention during wetland ecosystem succession. *Wetl. Ecol. Manag.*, **4**, 177-187 (1997).

Elser, J.J., M.E.S. Bracken, E.E. Cleland, D.S. Gruner, W.S. Harpole, H. Hillebradn, J.T. Ngai, E.W. Seabloom, J.B Shurin and J.E. Smith: Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.*, **10**, 135-1142 (2007).

Elser, J.J., E.R. Marzolf and C.R. Goldman: Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Can. J. Fish. Aqua. Sci.*, **47**, 1468-1477 (1990).

Gersberg, R.M., B. V. Elkins and C.R. Goldman: Role of aquatic plants in wastewater treatment by artificial wetlands. *Water. Res.*, **20**, 363-368 (1986).

Goldman, J.C., D.B. Porcella, E.J. Middlebrooks and D.F. Toerien: The effect of carbon on algal growth - Its relationship to eutrophication. *Water. Res.*, **6**, 637-679 (1972).

Güsewell, S., W. Koerselman and J.T. Verhoeven: Biomass N: P ratios as indicators of nutrient limitation for plant populations in wetlands. *Ecolo. Appl.*, **13**, 372-384 (2003).

Hecky, R.E. and P. Kilham: Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.*, **33**, 796-822 (1998).

Hey, D.L. and N.S. Philippi: Flood reduction through wetland restoration: The Upper Mississippi River Basin as a case history. *Restor. Ecol.*, **3**, 4-17 (1995).

Kadlec, R.H. and K.R. Reddy: Temperature effects in treatment wetlands. *Water. Environ. Res.*, **73**, 543-557 (2001).

Leonardson, L. and W. Ripl: Control of undesirable algae and induction of algal successions in hypertrophic lake ecosystems. In:

Hypertrophic ecosystems *Develo. Hydrobio.,* **2**, 57-65 (1980).

Mc Jannet, D., Wallace, J., Keen, R., Hawdon, A. and J. Kemei: The filtering capacity of a tropical riverine wetland: II. Sediment and nutrient balances. *Hydrol. Processes.,* **26**, 53-72 (2012).

Mitsch, W.J. and B.C. Reeder: Modelling nutrient retention of a freshwater coastal wetland: estimating the roles of primary productivity, sedimentation, resuspension and hydrology. *Ecol. Model.,* **54**, 151-187 (1991).

Muzaffar, S.B. and F.A. Ahmed: The effects of the flood cycle on the diversity and composition of the phytoplankton community of a seasonally flooded Ramsar wetland in Bangladesh. *Wetl. Ecol. Manag.,* **15**, 81-93 (2007).

O'Farrell, I., de Tezanos Pinto, P. and I. Izaguirre: Phytoplankton morphological response to the underwater light conditions in a vegetated wetland. *Hydrobiologia,* **578**, 65-77 (2007).

OECD: Eutrophication of Waters. Monitoring Assessment and Control. OECD, Paris (1982).

Ogawa, H. and J.W. Male: Simulating the flood mitigation role of wetlands. *J. Water. Resour. Plann. Manage.,* **112**, 114-128 (1986).

Pan, Y., R.J. Stevenson, P. Vaithiyanathan, J. Slate and C.J. Richardson : Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, USA. *Freshwater Biol.,* **44**, 339-353 (2000).

Pulatsu, S. and A. Topcu: Seasonal and vertical distributions of porewater phosphorus and iron concentrations in a macrophyte-dominated eutrophic lake. *J. Environ. Biol.,* **30**, 801-806 (2009).

Ralph, T.J. and K. Rogers: Floodplain wetlands of the Murray-Darling Basin and their freshwater biota. In: Floodplain Wetland Biota in the Murray-Darling Basin: Water and Habitat Requirements (Eds.: K. Rogers and T. Ralph). CSIRO Publishing, Collingwood, Melbourne., pp. 1-16 (2011).

Reddy, K.R., O.A. Diaz, L.J. Scinto and M. Agami : Phosphorus dynamics in selected wetlands and streams of the Lake Okeechobee Basin. *Ecol. Eng.,* **5**, 183-207 (1995).

Reynolds, C.S. and A.E. Walsby: Water-blooms. *Biol. Rev.,* **50**, 437-481 (1975).

Shardendu, S. I.: Dynamics of nitrogen in subtropical wetland and its uptake and storage by *Pistia stratiotes. J. Environ. Biol.,* **30**, 977-981 (2009).

Struyf, E. and D.J. Conley: Silica: an essential nutrient in wetland biogeochemistry. *Front. Ecol. Environ.,* **7**, 88-94 (2008).

Vincent, W.F., W. Wurtsbaugh, C.L. Vincent and P.J. Richerson: Seasonal dynamics of nutrient limitation in a tropical high-altitude lake (Lake Titicaca, Peru-Bolivia): Application of physiological bioassays1. *Limnol. Oceanogr.,* **29**, 540-552 (1984).

Vollenweider, R.A.: Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Technical Report. DAS/CSI/68.27. OECD, Paris (1968).

Vollenweider, R.A.: Input-output models with special reference to the phosphorus loading concept in limnology. *Schweiz. Z. Hydrol.,* **37**, 53-84 (1975).