Original Research

Roles of Nutrient Regime and N:P Ratios on Algal Growth in 182 Korean Agricultural Reservoirs

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Abstract

The principal aim of this study was to evaluate how nutrient regime and TN:TP ratios regulate algal chlorophyll in Korean agricultural reservoirs. We developed empirical models of log-transformed CHL-TN:TP ratio based on seasonality. Winter algal chlorophyll was highly influenced by the ratio of TN:TP ($R^2 = 0.78$, $p<0.01$) compared to chlorophyll-a in the spring ($R^2 = 0.38$, $p<0.01$), summer ($R^2 = 0.50$, $p<0.01$), and autumn ($R^2 = 0.44$, $p<0.01$), respectively. Regression analysis of log-transformed TN:TP ratios-TP and TN:TP ratios-TN on CHL-a showed that the ratio of TN:TP are highly influenced by the concentration of TP rather than TN. The ambient nutrient concentrations of the agricultural reservoirs showed positive linear relationships with water quality parameters of water temperature, pH, BOD, COD, DO, and TSS. Summer monsoon is an important factor that determines the nutrient regime, algal chlorophyll, and suspended solids in the reservoirs. Nutrient enrichment bioassays (NEBs) suggest that phosphorus was the primary limiting nutrient for algae production in the agricultural reservoirs. Analysis of trophic state index deviation (TSID) showed that P-limitation and biogenic turbidity (non-algal light limitation) were key regulating factors for algal growth in the reservoirs. Overall, our study suggested that the TN:TP ratio was the key regulating factor for algal biomass in the reservoirs.

Keywords: agricultural reservoirs, algal chlorophyll, nutrient enrichment bioassays, TN:TP ratio, trophic state index

Introduction

In the past two decades biotic diversity and trophic state of freshwater ecosystems have been mainly influenced by the total nitrogen:total phosphorus (TN:TP) ratio [1-2]. In an aquatic ecosystem, the TN:TP ratio is considered to be the key regulating factor of primary production (algal chlorophyll [3-5]), which ultimately controls zooplankton [6], invertebrate composition [7-8], and also fish trophic guilds [9-11]. The ratio of TN:TP is closely associated with land use activities that can directly influence the biotic components of an aquatic ecosystem [12].

Algal biomass growth (chlorophyll-a) in a freshwater ecosystem is controlled by various chemical and physical factors that include water temperature [4, 13], water column stability [14], ambient nutrient concentrations (particularly TN:TP ratio) [3, 15], low silica [16], high pH [17-18], lower deepwater light penetration [19-20], and the top-down grazing process of zooplanktons and fish [11,

21-22]. Among these factors the ratio of TN:TP has been considered the fundamental indicator of phytoplankton growth [3-4, 23].

The bimodal growth pattern of algal biomass depends on region and season [24]. For instance, in North America excessive nutrient enrichment of the reservoirs takes place in spring (May and June) due to heavy rainfall while spring TP influences algal chlorophyll during summer [25-27]. In contrast, in Asian reservoirs (e.g., South Korea) excessive nutrient loading caused by dense rainfall occurs in summer (July-August), and summer TP regulates algal growth in fall [28-29]. The TN:TP ratio alters the aquatic productivity of the ecosystem and has been widely used to assess the eutrophic condition of the waterbody and is regarded as the most limiting factor for algal growth. During last two decades, the TN:TP ratio has been used as an index to divide waterbodies into N- and P-limited categories. Generally, reservoirs have been regarded as limited by TN if the TN:TP ratio falls lower than 6, and TP if the ratio exceeds 16 [3-4].

The ratio of TN:TP plays a significant role in the growth of algal chlorophyll in American [30, 27] , European [31-32], and Asian [33-34] agricultural reservoirs. The nutrient dynamics of the Asian reservoirs – especially in South Korean – are affected by the summer monsoon [28, 35], which is an important determinant that controls the limnological characteristics (physical, chemical, and biological condition) of the freshwater ecosystem. During the monsoon period the concentration of TP had been increasing in the reservoirs due to intense rainfall, and a decreased concentration of TN is due to higher dilution [36]. The same scenario is being frequently observed in other tropical and subtropical Asian reservoirs, including those of Bangladesh [37], India [38], Nepal [39], and Sri Lanka [40].

In this study we observed how the TN:TP ratio and water quality parameters vary according to the season, and how the TN:TP ratio plays a key role in algal growth in Korean agricultural reservoirs. We developed many empirical models to see which one is the most important regulating factor for chlorophyll growth and also analyzed the trophic condition of the waterbody. We also conducted nutrient enrichment bioassays (NEBs) to know which one – N or P – is the most significant limiting factor for primary producers (Chlorophyll-a).

Materials and Methods

Sampling Sites and Sample Collection

The 182 agricultural reservoirs are located in four major watersheds of the Han, Nakdong, Geum, and Yongsan/Sumjin rivers of the Korean peninsula (36050' N, 1270 50'E). These reservoirs were formed from 1923 to 2011 for the major purpose of irrigating rice paddies. Mean depth of the reservoirs is 108 m and ranged from 2 to 700 m, indicating shallow lentic waterbodies. The basin area averaged 5.2 ha and ranged from 5.2 to 13,330

ha, indicating a small watershed. Most of the Korean agricultural reservoirs are monomictic and circular in shape. The storage capacity of the reservoirs (about 90%) is <1,000,000 m^3 [41-42]. The main characteristics of the Korean agricultural reservoirs are high watershed reservoir area ratios, shallow depth, unstable hydrodynamics, and eutrophic conditions [43]. More than 54% of the reservoirs are relatively old and were constructed 50 years ago. With aging reservoirs the sediment effect has a greater impact on water quality due to decreasing the volume and depth. Some previous studies have suggested that management of reservoirs is difficult due to the imbalanced rate of precipitation throughout the year and the high rate of soil erosion [43].

The sampling site in each reservoir was the deepest point of the limnetic zone every month during February-December 2013, and the samples were collected using a sampling bottle from the epilimnetic zone of the surface to 50 cm. Water samples were covered to prevent exposure to direct sunlight, stored in ice, and either preserved or analyzed in the laboratory within 12-36 hours. When we dealt with seasonal water quality, we used the terms of spring (March-May), summer (June-August), autumn (September-November), and winter (December-February) in describing temporal conditions.

Water Quality Parameters and Data Analysis

Secchi transparency (20 cm disk) was measured at the time of sample collection. Total nitrogen (TN), BOD, and COD were measured by the chemical testing method standardized by the Korean Ministry of the Environment [44]. Total phosphorus (TP) was determined using the
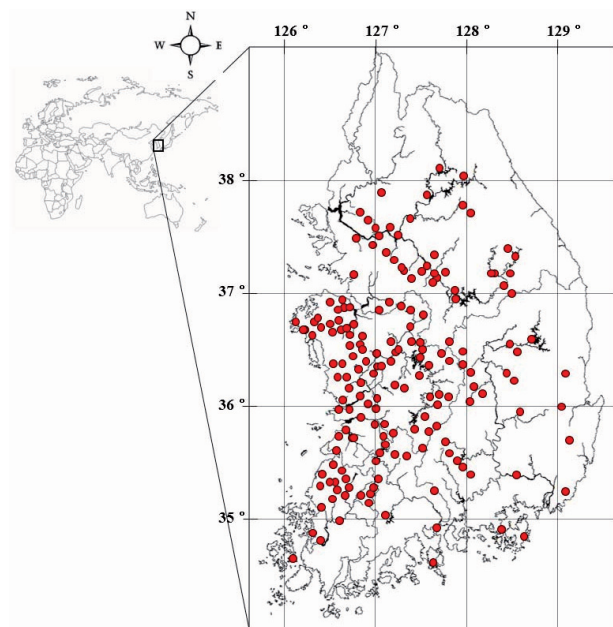

Fig. 1. Sampling sites of Korean agricultural reservoirs. Most of the agricultural reservoirs are located in western South Korea.
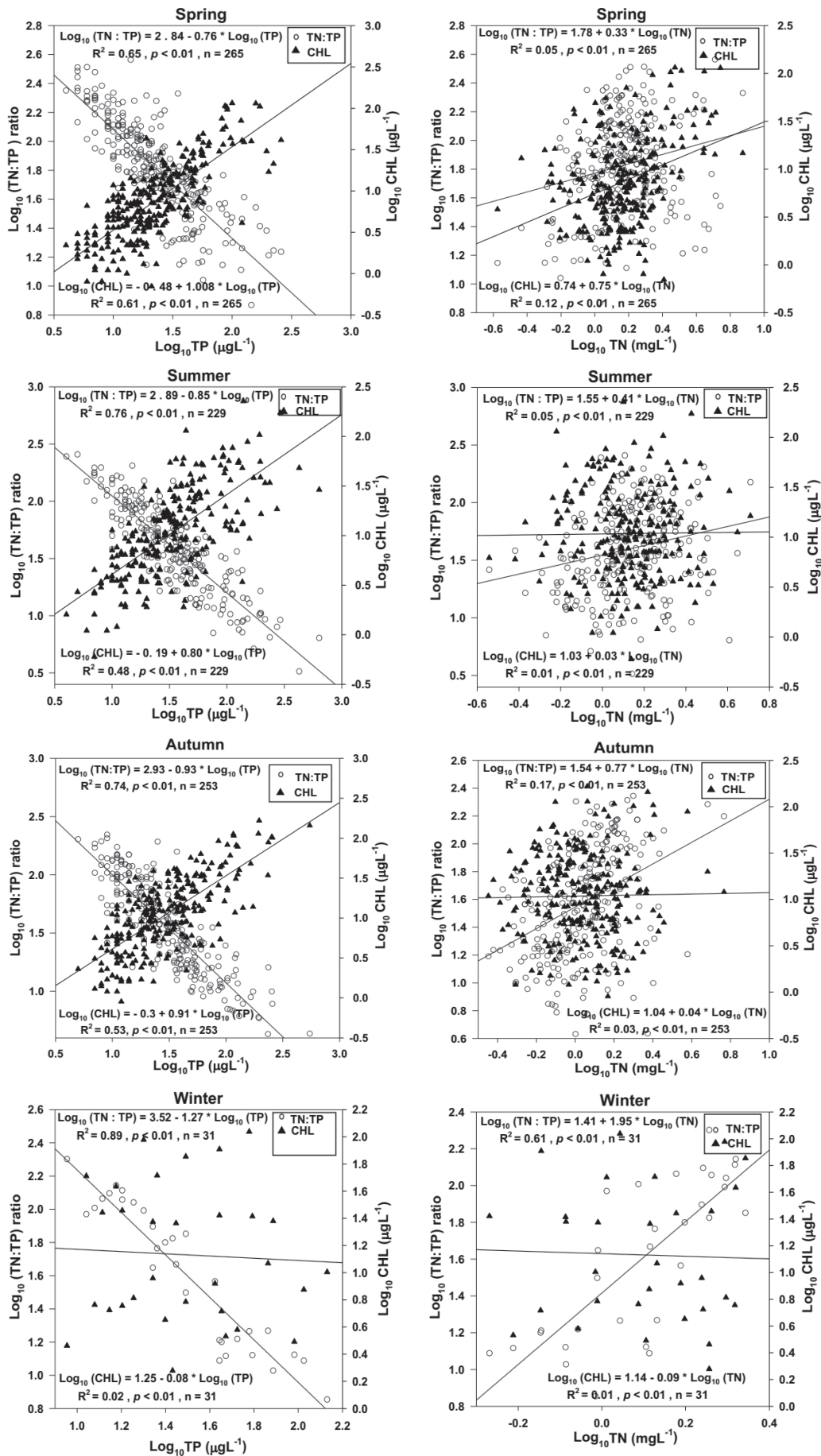
Fig. 2. Relationships of log-transformed TN:TP, CHL (μgL^{-1}), TP (μgL^{-1}), and TN (mgL^{-1}) based on season.
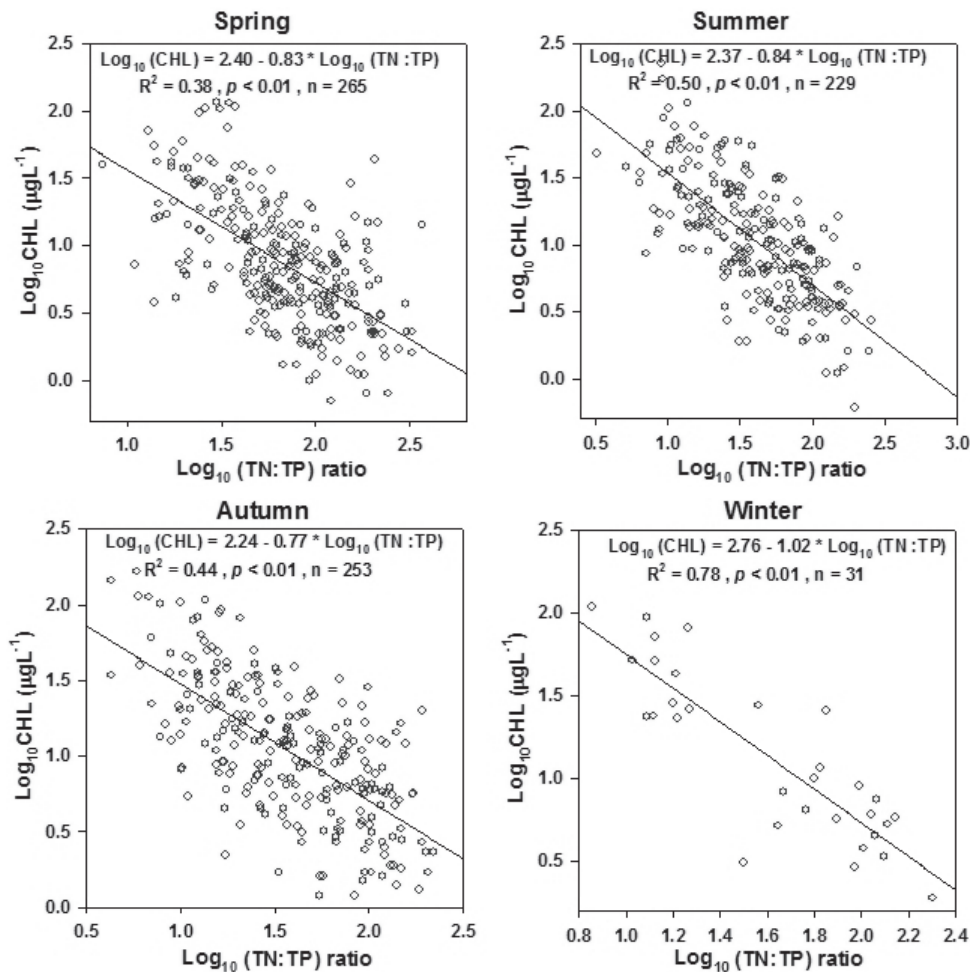
Fig. 3. Regression analysis of seasonal CHL-a (μgL^{-1}) on the Log_{10} (TN:TP) ratios.

ascorbic acid method after the chemical testing method standardized by the ministry [44]. Total suspended solids (TSS) filtered the water through pre-weighted Whatman GF/C filters which were then weighed after drying at 103°C for one hour. Chlorophyll-a (CHL) concentration was measured using a spectrophotometer (Bechman Model DU - 65) after extraction in hot ethanol [45]. Nutrient analyses were performed in triplicate and Chl was measured in duplicate.

Sigma plot software was used for statistical analysis (Systat Software Inc; USA, Version 10). The trophic state index (TSI) was calculated [46] using the following formula:

$$TSI \ (CHL, \mu gL^{-1}) = 10* \ [6 - (2.04 - 0.68 \ln (CHL)) \ / \ln2]$$
$$TSI \ (TP, \mu gL^{-1}) = 10* \ [6 - \ln (48/TP) \ / \ln2]$$
$$TSI \ (SD, m) = 10* \ [6 - \ln (SD) \ / \ln2]$$

Nutrient Enrichment Bioassays (NEBs)

To know the limiting characteristics of N or P of the Korean agricultural reservoirs we conducted nutrient enrichment bioassay. Each cubitainer contained 6L of the water and we categorized one control (C) and six treatment (T1, T2, T3, T4, T5, T6) conditions. For control

conditions we did not add any nutrients. We added 10, 20, and 40 μgL^{-1} of KH_2PO_4 in T1, T2, and T3, separately, which is known as P, 2P, and 4P in p concentration, respectively. Also, to T4 and T5 we added 1.5 and 3.0 mgL^{-1} of KNO_3 being N and 2N, accordingly. Lastly, to T6 we added 10 μgL^{-1} of KH_2PO_4 and 1.5 of KNO_3, known as N+P treatment. We settled each cubitainer on a water surface in a large container adding natural water and maintained the temperatures at 20±1°C.

Results and Discussion

Seasonal Empirical Relationship of TN:TP Ratios, Primary Productivity, and Nutrient Regime

The TN:TP ratios and values of chlorophyll (CHL, μgL^{-1}) are highly influenced by the concentrations of total phosphorus (TP, μgL^{-1}) and total nitrogen (TN, μgL^{-1}) depending on the seasonality of the Korean agricultural reservoirs (Fig. 2). The TN:TP ratios showed that it is strongly regulated by TP during four seasons like spring, summer, autumn, and winter, and the R^2 value is 0.65, 0.76, 0.74, and 0.89, respectively. Among this season, the TN:TP ratios were highly correlated with
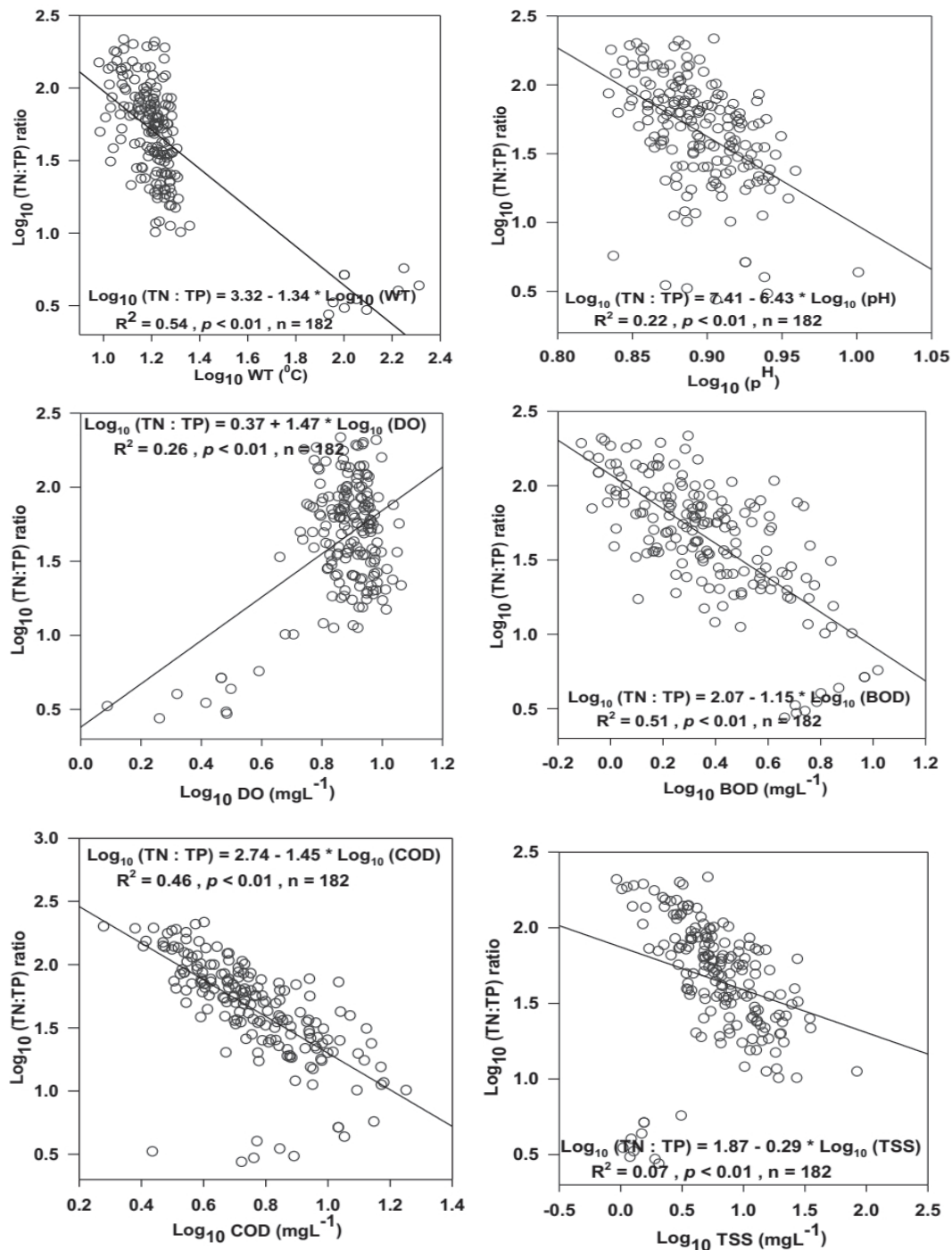
Fig. 4. Empirical regression model of log-transformed water temperature (ºC), pH, DO (mgL⁻¹), BOD (mgL⁻¹), COD (mgL⁻¹), and TSS (mgL⁻¹) on the TN:TP ratios.

TP throughout the winter season and controlled 89%. In contrast, the TN:TP ratios were less affected by the ambient concentrations of TN through the season but showed the remarkable relationship in winter, and the R^2 value was 0.61. This result agreed on the view that TN:TP ratios have a higher correlation with TP than TN in the lentic ecosystem [4, 47].

The primary production (concentration of CHL) of a waterbody largely depends on the concentration of nutrient regime and the limiting factor is TP and TN. Values of CHL were extremely influenced by TP through spring ($R^2 = 0.61$) and suddenly were reduced in the summer ($R^2 = 0.48$) by the high flushing rate of water, inorganic turbidity, and then getting better in the autumn ($R^2 = 0.53$). The concentration of CHL is a little bit affected by TN throughout the year. The relationship of CHL-TP and CHL-TN based on the season reported here relies on the fact that primary productivity of a lentic ecosystem is greatly controlled by the availability of TP – not by TN [2].
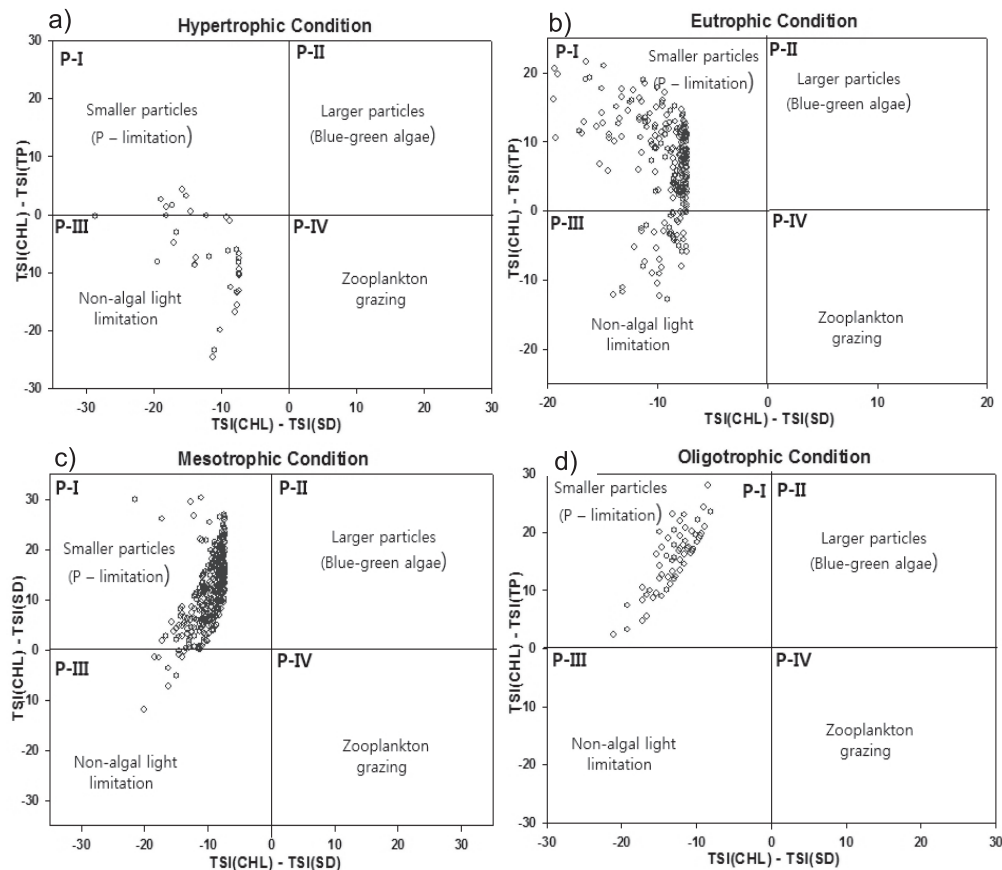
Fig. 5. Trophic state index deviation on the various trophic conditions of hypertrophic, eutrophic, mesotrophic, and oligotrophic reservoirs.

Most of the Korean agricultural reservoirs were constructed more than 50 years ago, and owing to this fact internal loadings of the nutrients were greater, which accelerates the eutrophication process of the reservoirs depending on the season. Previous studies found that primary productivity of the reservoirs were greater due to aging of the reservoirs [42]. Most of the nutrients (N, P) come from the paddy field in the reservoirs, and the water body becomes eutrophic. The eutrophication process was fast in the reservoirs due to the shallow waters and small basin areas. Some earlier studies suggested that primary productivity of the reservoirs were high due to shallow waters, which directly regulate the internal loading of the nutrients [48] and strongly support our findings. Kalff (2001) suggested that nutrient loading increased with high watershed reservoir area ratios and lead to the possibillity of eutrophication [49]. A similar scenario was observed in our study.

Analysis of Seasonal Variation of Primary Productivity on the Basis of Ambient Nutrient Concentrations

The TN:TP ratios were a good predictor for the primary production of the Korean agricultural reservoirs (Fig. 3). The relationship of CHL-TN:TP showed that it was more strongly correlated in the winter ($R^2 = 0.78$) than the spring, summer, and autumn ($R^2 = 0.38$, 0.50, and 0.44,

individually). The prediction of primary productivity in winter was high ($R^2 = 0.78$) in comparison with all other seasons. The high CHL yields were due to nutrient (N, P) supplies in late monomictic overturn during December to February. During winter the water level lowers and the nutrient concentration increases, which directly determines the primary productivity of the reservoirs. Kim et al. (2001) found that internal loadings of nutrients from the water column during winter in Korean reservoirs had happened, which supports our present study [50].

During summer and winter TN:TP was the better forecaster for CHL than TP, while in spring and autumn TP was a better indicator than TN:TP for the CHL concentrations. This strongly supports the idea that TN:TP ratios determined the primary productivity of the waterbody [33]. According to Jung et al. (2016), the primary productivity (CHL-a) of the Korean reservoirs is highly dependent on TN:TP ratios, which is in line with the findings of present study [35]. During summer the TN:TP ratio was highest in American reservoirs and had a great impact on algal chlorophyll [27].

Analysis of TN:TP Ratios with other Water Quality Parameters

TN:TP ratio is a good surrogate variable of ambient nutrient concentrations of the Korean watershed

[33]. The TN:TP was highly influenced by water temperature (WT, °C), biological oxygen demand (BOD, mgL^{-1}), chemical oxygen demand (COD, mgL^{-1}), dissolved oxygen (DO, mgL^{-1}), hydrogen ion concentration (pH), and total suspended solids (TSS, mgL^{-1}; Fig. 4). The TN:TP ratio was intensely controlled by water temperature and the R^2 value is 0.54. BOD and COD also are an important factor for regulating the ambient nutrient concentrations of the reservoirs. The R^2 values are 0.51 and 0.46, correspondingly.

High flushing rate in the water increases the DO level, and TN:TP had a positive linear relationship with DO ($R^2 = 0.26$). During summer, the value of TSS and pH increased due to heavy rainfall and affected the TN:TP ratios of the reservoir. Stefanie et al. (2013) stated that water temperture has a direct impact on ambient nutrient concentrations [13]. Long-term analysis of water quality parameters (2003-14) in China by Jiang et al. (2016) proposed that the nutrients of the freshwater ecosystem are greatly influenced by the COD,
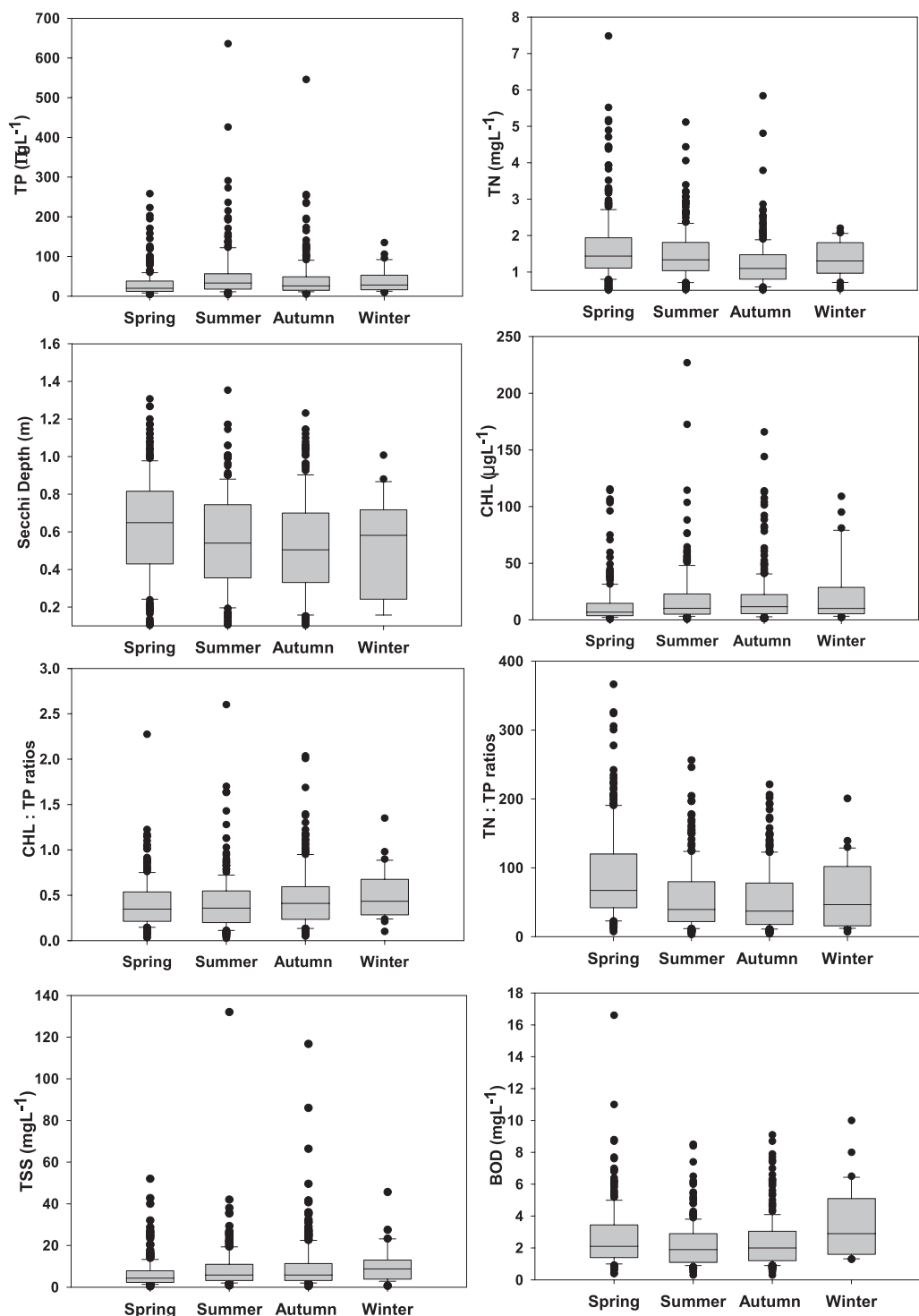

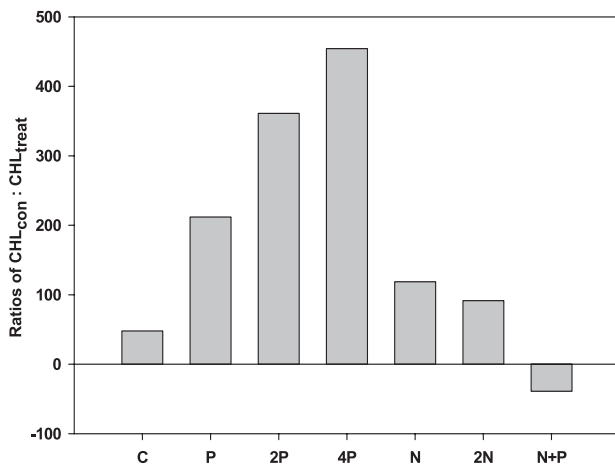Fig. 6. Seasonal variations of water quality parameters.

Fig. 7. Nutrient enrichment bioassays (NEBs) conducted to identify nutrient limitations (P, N, or N+P) to the algal response were expressed as the ratios of CHL_{con} (CHL-a concentration in the control conditions) and CHL_{treat} (CHL-a concentration in the treatment conditions).

BOD, TSS, and pH, which corroborates the findings of our study [51].

Analysis of Trophic State Index Deviation (TSID) of the Reservoirs

Analysis of trophic state index deviation [46] disclosed that P-limitation and biogenic turbidity was dominant in Korean agricultural reservoirs (Fig. 5). After examining the hypertrophic condition, only 9.2% of observations fell in Phase I, which indicates that smaller particles (i.e., P-limitation) occurred, and for the majority of the observations about 90.8% were gone in Phase-III, meaning that non-algal light limitation (i.e., biogenic turbidity) occurred. In eutrophic conditions smaller particles were notable than the non-algal light limitation and the estimation percentages were 82.75 and 17.25, accordingly. The 97.2% observations revealed that P-limitation and a very little bit showed that non-algal light limitation (2.8%) happened in mesotrophic conditions. P-limitation was extremely prominent in oligotrophic conditions, and the 100% observation fell in Phase-I. It strongly considered that phosphorus and biogenic turbidity was the key factor regulating the trophic state of the Korean reservoirs [52]. In USA the analysis of TSID of four major lakes of the State of Mississippi showed dominant biogenic turbidity and a little bit phosphorus limitation, which means that most of the lakes were in hypertrophic condition [53].

Seasonal Variation of Water Quality Parameters

The summer monsoon influenced the nutrient regime, primary productivity and suspended solids in the Korean agricultural reservoirs (Fig. 6). Concentrations of TP suddenly increased in the summer period due to a high amount of runoff water from farmland. Values of CHL and TSS also rose during the summer because of intense rain and a high amount of runoff. The concentration of TN was highest in the spring and abruptly decreased during the summer due to dilution of TN by rainwater. The mean Secchi depth value was lowest in autumn. The CHL:TP ratios were highest in summer whereas TN:TP ratios peaked in spring and sharply declined during summer due to intense rainfall. These results agree well with the viewpoint that summer monsoon greatly determined the ambient nutrient concentrations, algal chlorophyll, and total suspended solids [36]. Zhang et al. (2016) observed similar monsoon patterns in China and South Korea, leading to a change of physico-chemical water quality of agricultural reservoirs [54].

Nutrient Enrichment Bioassays (NEBs) *in-situ*

Nutrient enrichment bioassays (NEBs) indicated that phosphorus (P) was the most crucial factor for algal growth (Fig. 7). In the NEB experiment, higher enrichments of phosphorus result in greater algal growth. The algal response was in the top position in 4P treatments (ratios of CHL = 454.24). The algal growth was three times the 2P and 2N treatments (ratios of CHL = 361.01 and 91.52, individually). In P and N treatments the ratios of CHL were higher in P than N treatments, and the values were 211.86 and 118.64, separately. The N+P treatments showed negative growth of algae over control conditions (ratios of CHL = -38.98 and 47.89, respectively). This experiment supports P being the most dominant factor for algal growth [28]. Taofikat Abosede Adesalu (2013) conducted an NEB experiment and saw that P was the most important factor for algae growth [55].

Short-term Analysis of Nutrient Stimulation Experiments (NSEs)

Algae growth was more influenced by P treatments than N treatments (Fig. 8). The concentration of CHL increased with enrichment by the P treatments. The CHL concentration exhibited an increasing trend with the passage of time in P, 2P, 4P, and N+P treatments. In C, N, and 2N treatments the value of CHL did not show any kind of positive relationship with time. It agreed that phosphorus was the limiting factor for algal growth in the waterbody [56]. Ostman et al. (2016) showed that chlorophyll was highly influenced by the nutrients, especially phosphorus rather than nitrogen, meaning that phosphorus is the key regulating factor for algae growth in any water body [57].

Conclusions

The present research concluded that the primary production (chlorophyll-a) of agricultural reservoirs is controlled by the TN:TP ratio. The ratio of the ambient
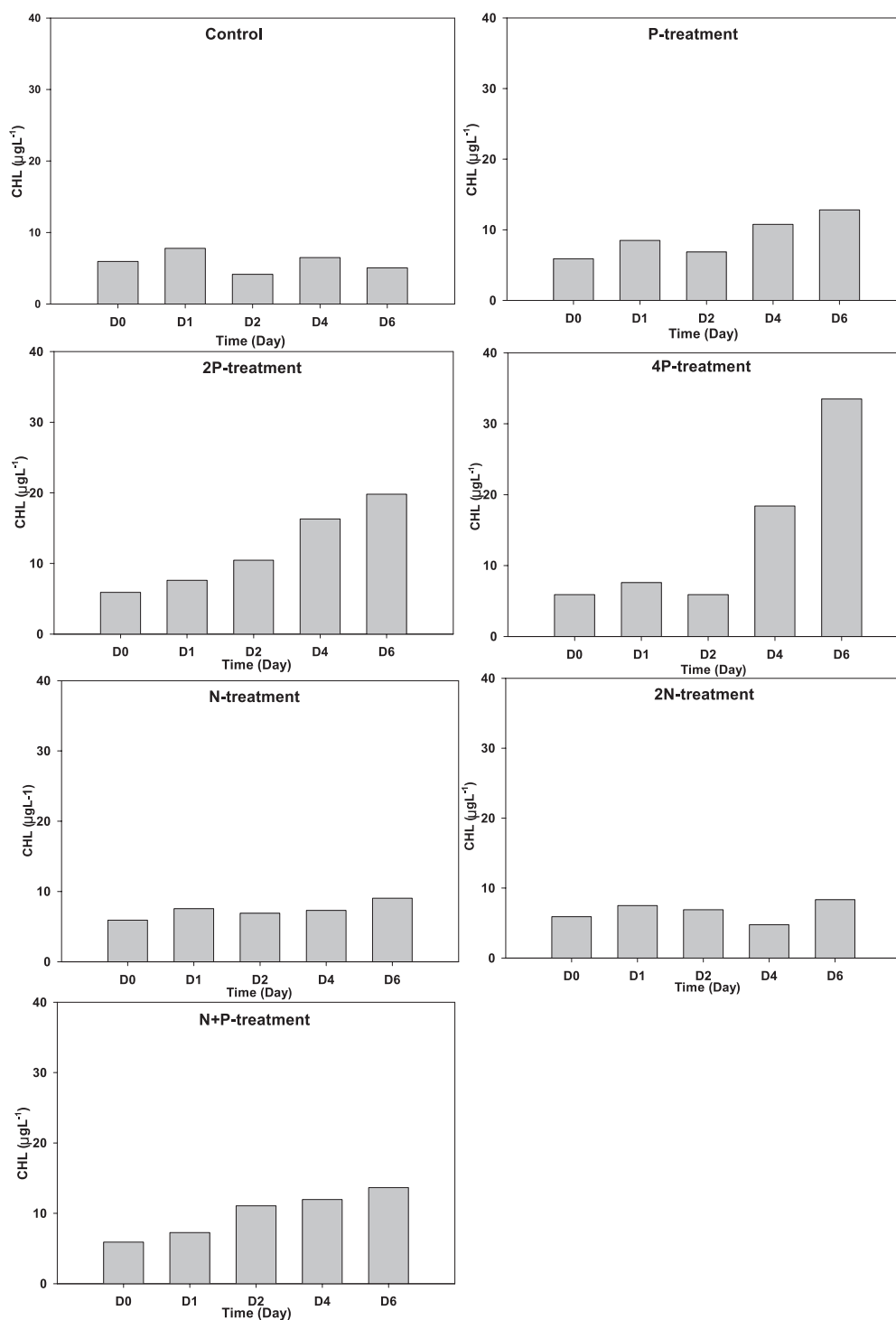
Fig. 8. Algal response as chlorophyll-a concentrations in the control, P-treatment, 2P-treatment, 4P-treatment, N-treatment, 2N-treatment, and N+P-treatment in nutrient enrichment bioassays (NEBs).

nutrients vary in season and are influenced by the summer monsoon. TP was the most important factor, which regulates the nutrient ratio in the aquatic ecosystem. Trophic state index deviation analysis stated that most of the Korean agricultural reservoirs are P-limited and non-algal light limitation is frequent. After the nutrient enrichment bioassay analysis we concluded that phosphorus was the main limiting factor for algal growth in Korean agricultural reservoirs.

Acknowledgements

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