Nutrients structure changes impact the competition and succession between diatom and dinoflagellate in the East China Sea

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HIGHLIGHTS

- The competition between diatom and dinoflagellate were evaluated through field and indoors experiments in the ECS.
- Low Si/N caused by Three Gorges Dam construction could reduce relative abundance of diatom and benefit dinoflagellate.
- Large DIN flux from anthropogenic activities and induced high N/Si and N/P impel the eruption of dinoflagellate.
- D_t is a preferable parameter to represent the relative superiority of two algae.

GRAPHICAL ABSTRACT
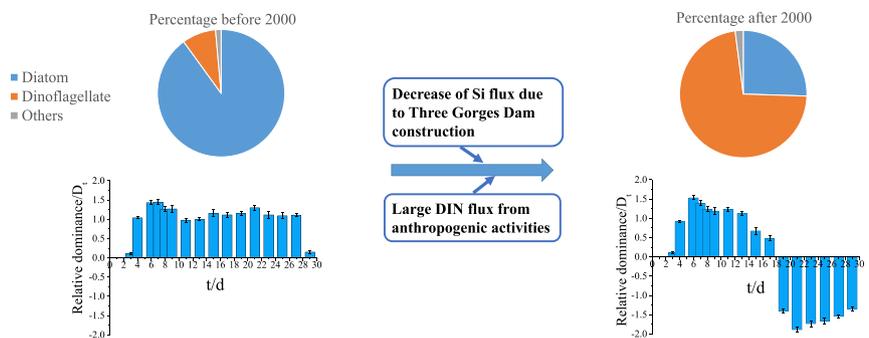
ABSTRACT

Nutrients variations caused by anthropogenic activities alter phytoplankton community interactions, especially competition and succession between two algal species. East China Sea experiences annual successions of *Skeletonema costatum* and *Prorocentrum donghaiense* and large-scale blooms of *P. donghaiense*. In this study, the growth and competition responses of *S. costatum* and *P. donghaiense* to different inorganic nutrients structure were evaluated through field and indoors experiments to further understand the nutrients mechanism of these events. Results showed that low Si/N ratio (Si/N < 1) and high N/P (>50) possibly accelerated P. donghaiense outbreak and decreased Si/N caused by low Si concentration could speed up *S. costatum* decay. Excessive DIN also accelerated blooms dominated by *P. donghaiense* (D_t up to −3) when *S. costatum* perished. Increased DIN loads from anthropogenic activities were possibly responsible for the changes in phytoplankton communities and dinoflagellate outbreak when Si concentration decreased as a result of governmental control efforts. With effective management practices for Si and P reductions worldwide, managers should be aware of the negative implications of unsuccessful management of system N loading because N may significantly alter the composition and ecosystem of phytoplankton communities.

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1. Introduction

Harmful Algal Blooms (HABs) have emerged as a global phenomenon affecting coastal areas (Granéli et al., 2008). Large-scale of dinoflagellate blooms, which increasingly erupted in recent years (Park et al., 2013; Lim et al., 2014; Giussani et al., 2015; Menden-Deuer and Montalbano, 2015), have progressively replaced diatom as dominant harmful algae and severely threatened global coastal ecosystem and economies (García-Camacho et al., 2007; Fuentes-Grünewald et al., 2015; Gutierrez- Lopez-Rosales et al., 2015; Gutierrez-Mejia et al., 2016). This phenomenon was traditionally thought to occur as a result of interspecific competition ascribed to changes in environmental factors, such as temperature, salinity, irradiance, DO, and disruption of chemical release (Hong and Xu, 2014; Van der Lingen et al., 2016). For example, increased CO_2 alone or increased CO_2 and temperature stimulate *Heterosigma akashiwo* to become more dominant than *Prorocentrum minimum* (Fu et al., 2008). Low illumination intensity reduces the dominance of *Heterosigma akashiwo* with respect to *Skeletonema costatum* (Xu et al., 2010). Algal dominance is maintained by releasing allelochemicals, for instance, *Chaetoceros curvisetus* inhibits *S. costatum* growth through allelopathy (Zhang et al., 2014). Coastal dinoflagellate blooms are also restricted by warm waters (Ryan et al., 2008; Pitcher et al., 2010; Kibler et al., 2015; Van der Lingen et al., 2016). Nutrient-rich waters can stimulate dinoflagellate blooms, although dinoflagellate can endure low nutrient concentrations (Charles et al., 2005). Sea water composition influences the relative abundance of *Prorocentrum* and *Skeletonema* sp. because of competition for resources (Hong and Xu, 2014). Under different nutrients conditions, these algae employ different selected adaptive strategies. For instance, *Skeletonema* sp. yields a higher nutrient uptake rate than *Prorocentrum* sp. do, and *Prorocentrum* sp. are K-selected, that is, it has adapted to low trophic conditions (Zhao et al., 2009; Liang et al., 2014). The two kinds of algae also assimilate nutrients in different proportions. In general, cultures with low N/P ratios benefit the growth of diatoms and cultures with low Si/N ratios favor the growth of dinoflagellates (Papush and Danielsson, 2006; Gieskes et al., 2007). Considering these phenomena, we hypothesized that changes in nutrient compositions likely influence the relative abundance of algae. Hence, the effects of nutrient structural changes on the competition and succession between diatom and dinoflagellate were evaluated in the East China Sea (ECS).

In ECS, *P. donghaiense* blooms occurred in late spring and summer (May and June) from 2002 to 2013 (Lin et al., 2008; Ma et al., 2013). These blooms are associated with changes in environmental factors, such as temperature increase (Wang et al., 2008; Liu et al., 2013) and eutrophication caused by anthropogenic perturbations along the Changjiang (Yangtze) River, which resulted in increasing of N concentration and P concentration (Zhang et al., 2006a, b, 2007; Li et al., 2014). SiO_3-Si flux was mainly influenced by the construction of the Three Gorges Dam, and the mean SiO_3-Si concentration decreased from 35 $\mu mol \cdot L^{-1}$ in 1959 to 18 $\mu mol \cdot L^{-1}$ in 2010 (Li et al., 2007). Phosphate supplied by Taiwan Warm Current is also an important factor that stimulates the *P. donghaiense* outbreak (Zhou et al., 2001, 2003). Anomalous nutrient variation during *P. donghaiense* and *S. costatum* blooms has also been extensively explored through field investigations since the 1980s. Results revealed that DIN/Si and DIN/P abruptly increase because of increased DIN flux and decreased Si flux (Harrison et al., 2010; Jin et al., 2010) in ECS areas where HABs frequently occur. This observation suggests that excessive DIN and increasing N/P may be responsible for the changes in phytoplankton community structures in ECS (Wang, 2006). Nutrient compositions also significantly differ during the outbreak and eradication of different phytoplankton species (Li et al., 2014). In terms of individual algal behaviors related to nutrient changes, *P. donghaiense* is more adaptable to low phosphate conditions than *S. costatum* significantly (Zhao et al., 2009).

Field nutrient changes during blooms and individual algal responses to nutrients have been widely explored, however, the effects of anthropogenic activities, especially induced nutrients structural variations, on the competition between *P. donghaiense* and *S. costatum* have been rarely investigated. With an enhanced understanding of competition, ecological factors influencing the gradual domination of dinoflagellates might be fully elucidated. Therefore, our study aimed to evaluate the effects of anthropogenic activities on phytoplankton community. Changes in the competitive response of *P. donghaiense* and *S. costatum* to increase inorganic N and decrease inorganic Si throughout the growing season were examined through mixed culture biotechnology under field and indoor conditions.

2. Materials and methods

2.1. Nutrient enrichment experiments

Blooms in ECS persist from April to July every year. *S. costatum* dominates the phytoplankton community biomass in April and early May, comprising approximately 90% of the biomass. *P. donghaiense* bloom is dominant in mid-to-late May. Station Za1 is a typical representative of HABs in ECS as shown in Fig. 1. Upon sampling on May 12, 2011 in Za1 (29°33′N, 122°28.5′E), the phytoplankton community was co-dominated by *S. costatum* and *P. donghaiense*. The water samples were used for short-dated field mesocosm experiments. Throughout the whole period of culturing in station A, surface water temperature ranged from 16.5 to 23.1 °C, and salinity and pH were roughly constant (mean: 33.4 ± 0.2 and 8.46 ± 0.04, respectively). DIN average concentration ranged from 5–25 $\mu mol \cdot L^{-1}$ (Cheng and Li, 1992; Li et al., 2013) and the average PO_4-P concentration was approximately 0.8 $\mu mol \cdot L^{-1}$ (Chen et al., 2004; Wang, 2006), and the mean SiO_3-Si concentration in ECS ranged from 18–35 $\mu mol \cdot L^{-1}$ (Li et al., 2007). The following field and lab experiments were conducted based on these concentrations. The environmental conditions and culturing media parameters of indoor experiments were similar to field surroundings (temperatures 20.0 ± 0.2 °C, light levels 70 $\mu mol\,m^{-2}\,s^{-1}$, photoperiods 14:10 h).

2.1.1. Field experiment

Short-dated field mesocosm experiments were conducted to evaluate the impact of increased inorganic N concentration on the competition and succession between *S. costatum* and *P. donghaiense* in the ECS. From May 13 to 30, 2011, a total of 12 mesocosm experiments were conducted on the coastal station A (Fig. 1). A total of 1000 L clear PE enclosure containers ($n = 12$) were filled with 750 L original surface seawater containing the natural phytoplankton community from the station Za1 on May 12, 2011. Four nutrient treatments, in sets of triplicate, were tested as follows: the control contained original surface water (Control, 10 $\mu mol \cdot L^{-1}$ DIN, 1.0 $\mu mol \cdot L^{-1}$ P and 7.1 $\mu mol \cdot L^{-1}$ Si), and the experimental cultures were added to a final stationary concentration of Si (15 $\mu mol \cdot L^{-1}$) with exponential N concentration 10 $\mu mol \cdot L^{-1}$ (A1), 20 $\mu mol \cdot L^{-1}$ (A2) and 30 $\mu mol \cdot L^{-1}$ (A3). When *S. costatum* blooms were about to disappear (approximately day 13), N and Si treatments were all resupplied with the initial content whereas P was resupplied with a fixed concentration (0.8 $\mu mol \cdot L^{-1}$) in all experimental cultures.

2.1.2. Lab experiment

To make a comprehensive exploration about the effects of inorganic nutrients on the competition and succession between *S. costatum* and *P. donghaiense*, two series of experiments similar to field experiments were conducted in the laboratory. A series of four experimental cultures (group M) were conducted to evaluate the impacts of increased N loading, decreased Si loading and N/Si, which were designed as follows: all P treatments were amended to 0.8 $\mu mol \cdot L^{-1}$, and three experimental cultures were amended to a final N concentration of 22.0 $\mu mol \cdot L^{-1}$ with exponential Si concentration of 15.0 $\mu mol \cdot L^{-1}$ (M1), 30.0 $\mu mol \cdot L^{-1}$ (M2) and 45.0 $\mu mol \cdot L^{-1}$ (M3), and the last experimental culture was supplied to a final N concentration of 44.0 $\mu mol \cdot L^{-1}$ (M4) with Si
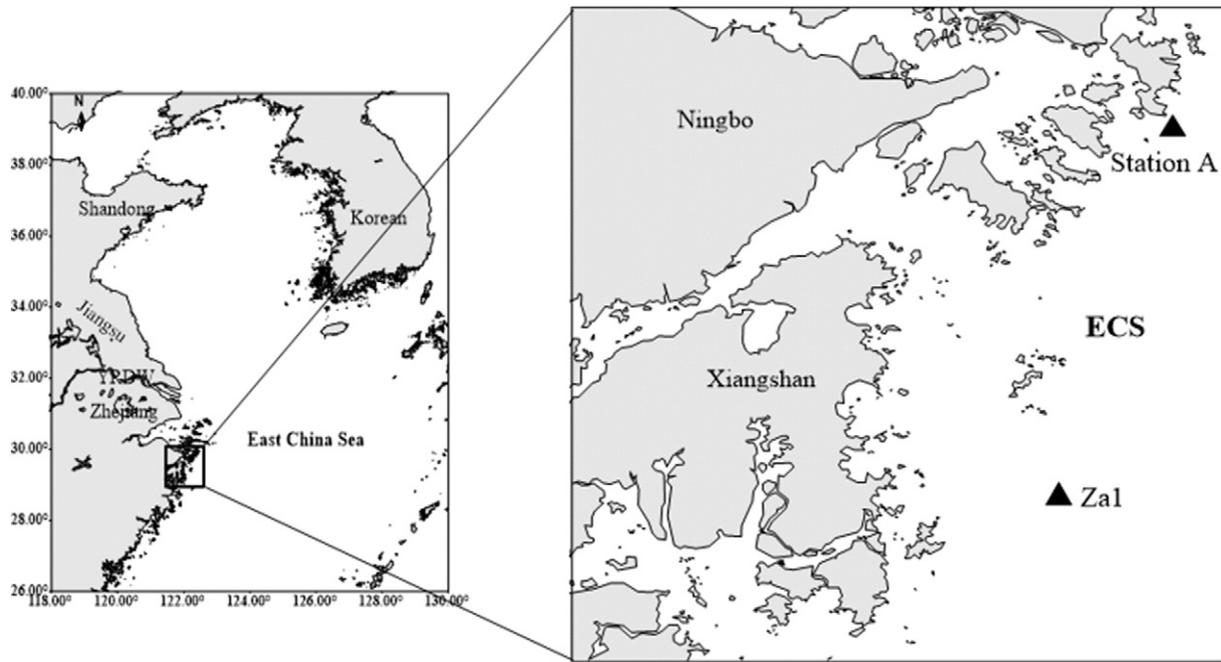
Fig. 1. The field experiment station (A) and sample station (Za1) in the ECS.

concentration 30.0 μmol·L^{-1}. When *S. costatum* nearly faded (approximately on day 12), all experimental cultures were resupplied with 22.0 μmol·L^{-1} N, 0.8 μmol·L^{-1} P and 15 μmol·L^{-1} Si. Another series of three experimental cultures (group N) were conducted to study the impacts of increased P and N loading in the medium with higher N/Si. All cultures were amended to a final Si concentration of 11 μmol·L^{-1}, two experimental cultures were added to a final P concentration of 0.8 μmol·L^{-1} with N concentration of 22.0 (N1) and 44.0 μmol·L^{-1} (N2), and another experimental culture was amended to a final P concentration of 1.5 μmol·L^{-1} with 44.0 μmol·L^{-1} N (N3). When *S. costatum* almost deteriorated (approximately on day 14), all experimental cultures were amended with 22.0 μmol·L^{-1} N, 0.8 μmol·L^{-1} P and 11 μmol·L^{-1} Si. Experimental cultures were conducted in 3 L PE bottles containing 2 L autoclaved 0.22 μm filtered natural seawater collected from the station Za1. To commence the experiments, 10^{-2} cells mL^{-1} of *S. costatum* and *P. donghaiense* were mixed into all experimental culture media.

2.2. Sample collection and analysis

Biomass and phytoplankton community compositions in all cultures were measured daily under a light microscope (Leica DM4000B) using a haemocytometer. To clarify the relative dominance of each of the two algae, the dominance index D_t was suggested as the following formula according to the algal density.

$$D_t = \log(C_s/R_s) - \log(C_p/R_p) \qquad (C_s \neq 0 \,\&\, C_p \neq 0)$$

$$D_t = \log(R_p/C_p) \qquad (C_s = 0 \,\&\, C_p \neq 0)$$

$$D_t = \log(C_s/R_s) \qquad (C_s \neq 0 \,\&\, C_p = 0)$$

where C_s is the cell density of *S. costatum*, C_p is the cell density of *P. donghaiense*, and R_s (5×10^6 cells·L^{-1}) and R_p (5×10^5 cells·L^{-1}) are the reference densities of *S. costatum* and *P. donghaiense* blooms respectively, which were suggested by Zhang et al. (2006a, b) on the basis of field experiments. When D_t is positive, *S. costatum* is the dominant algal species, otherwise *P. donghaiense* is the dominant algal species. In

addition, a higher absolute D_t implies that the dominant algae gained a highly advantageous position.

Water samples were decanted and filtered daily (0.45 μm Whatman glass fiber filters) from the raw water samples in the cultures and immediately spectrophotometrically analyzed for the concentrations of DIN (NO$_3$-N + NO$_2$-N + NH$_4$-N), PO$_4$-P and SiO$_3$-Si according to Zhu (2006). The standard deviations from the spectrophotometric analysis for NO$_3$-N, NO$_2$-N, NH$_4$-N, PO$_4$-P and SiO$_3$-Si were ±0.3 μM, ±0.02 μM, ±0.1 μM, ±0.02 μM and ±0.2 μM respectively (Grasshoff et al., 1999).

Maximum nutrients uptake content per volume (N) and maximum utility rate (U) were calculated following the formula.

$$N = (N_{max} - N_{min})/V$$

$$U = (N_{max} - N_{min})/N_{max}$$

where N_{max} is the maximum content of nutrients in the media, N_{min} is the minimum content of nutrients and V is the medium volume.

2.3. Statistical analysis

In order to explore the effects of nutrient structures on the growth and superiority degree of *S. costatum* and *P. donghaiense*, average of biomass and D_t were compared among different nutrient conditions (one-way ANOVA). To test possible relationship between relative abundances of two algae and Si/N ratios during succession of diatom to dinoflagellate, a simple correlation analysis was carried out. These analyses were performed with statistical significance of 95% confidence level.

3. Results

3.1. Phytoplankton growth

Four experiments were conducted *in situ* and seven were conducted indoors. The growth of phytoplankton was obtained through the biomass counted by a microscope. Both conditions showed similar overall responses in algal growth, whereas each alga exhibited different responses to different nutrient additions in both conditions (Figs. 2, 3

and 4). In the original seawater, the phytoplankton community was co-dominated by *S. costatum* and *P. donghaiense*. Adding nutrients strongly stimulated the growth of *S. costatum* which peaked on day 5 in all cultures in the initial culturing stage. *P. donghaiense* initially had a lower algal density in all experimental cultures. After resupplying nutrients, different nutrient treatments yielded different biomass of *S. costatum* and *P. donghaiense*.

Algal growth responses to different DIN concentrations in field conditions were compared as shown in Fig. 2. *S. costatum* maxi-density in the media with higher DIN concentration only increased by 15.3% relative to lower DIN concentration (Fig. 2A and B) and additional DIN did not stimulate the growth of *S. costatum* when Si/N was <1 (Fig. 2B and C). Moreover, when Si/N was lower than 1, addition of Si significantly stimulated the growth of *S. costatum*, which indicated that the 68.8% increase in *S. costatum* maxi-density in the media with higher Si concentration relative to control ($p < 0.05$, Fig. 2A and D). *P. donghaiense* biomass did not satisfy the criterion of HABs (5×10^2 cells·mL^{-1}) in the initial culturing stage. After resupplying nutrients, *P. donghaiense* started to erupt and biomass reached >3-fold (Fig. 2C and D) in the culture with 40 μmol·L^{-1} DIN relative to control. However, *S. costatum* biomass did not satisfy the criterion of algal blooming (5×10^3 cells·mL^{-1}) in this stage.

Fig. 3 showed algal growth changes in the medium with different Si concentrations (Fig. 3A, B and C) and DIN concentrations (Fig. 3B and D) in the indoor experimental group M. In the initial culturing stage, *S. costatum* maxi-density in the culture which had lower Si/N (Si/N < 1) due to decreasing of Si concentration, decreased by 47.9% relative to control (Fig. 3A and B), whereas further addition of Si did not stimulate *S. costatum* growth relative to control (Fig. 3B and C). *S. costatum* maxi-density in the culture with a higher DIN concentration increased by 22.6% relative to control (Fig. 3B and D). *P. donghaiense* developed a small maxi-biomass, which was significantly lower than that of *S.*

costatum (Fig. 3). After being resupplied with nutrients, *S. costatum* erupted again and reached a higher biomass (approximately 25×10^4 cells·mL^{-1}) on day 16 (Fig. 3B and C) in the culture with 45 μmol·L^{-1} Si and in control. *P. donghaiense* erupted in all cultures except in the culture with 45 μmol·L^{-1} Si, in which biomass of *P. donghaiense* still decreased. Moreover, *P. donghaiense* maxi-biomass in the culture with 44 μmol·L^{-1} DIN increased to 4-fold relative to control (Fig. 3B and D).

Another set of indoor experimental results in group N were presented in Fig. 4. In the initial stage, *S. costatum* maxi-density in the culture with 44 μmol·L^{-1} DIN increased by 47.6% relative to control, whereas *P. donghaiense* developed a small biomass. After being resupplied with nutrients, did *S. costatum* erupt again with a maxi-biomass of 12.1×10^4 cells·mL^{-1} in the culture with 1.5 μmol·L^{-1} P. However, *P. donghaiense* erupted in all cultures although with lower biomass in control. Moreover, *P. donghaiense* maxi-biomass amounts in the cultures with 44 μmol·L^{-1} DIN and 0.8 μmol·L^{-1} P increased 36-fold relative to control, whereas maxi-biomass amounts in the cultures with 44 μmol·L^{-1} DIN and 1.5 μmol·L^{-1} P only increased 16-fold.

3.2. The relative abundance of diatom and dinoflagellate

The relative abundance of diatom and dinoflagellate was conveniently described by relative dominance parameter D_t and variable D_t response was shown in Figs. 5, 6 and 7. D_t was greater than zero before resupplying nutrients in all cultures and reached the maxi-value on day 4–7, which indicated that *S. costatum* dominated phytoplankton communities. In both field and indoors experiments, maxi-D_t in the culture that initially had low Si/N (Si/N < 1) due to lower Si concentration (Figs. 5D and 6A) was significantly less than other cultures ($p < 0.05$), which suggested that decrease of Si concentration would reduce relative superiority of *S. costatum*. However, when initial Si concentration
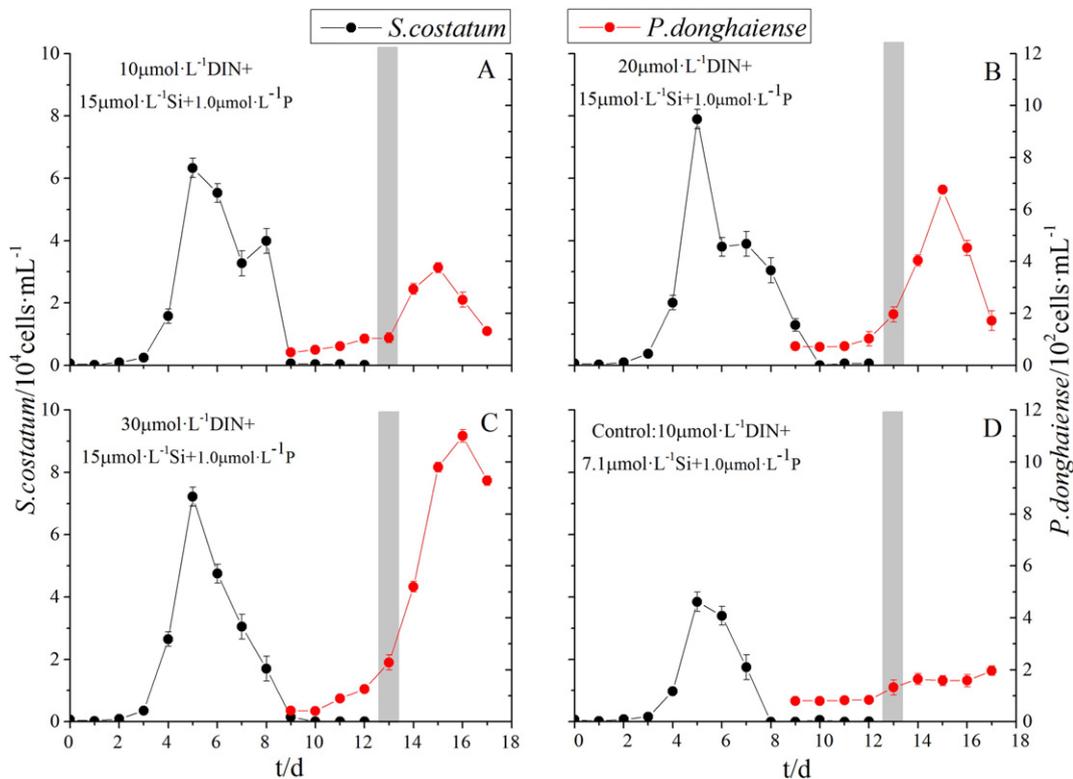
Fig. 2. The changes of diatom *S. costatum* (black spot) and *P. donghaiense* (red spot) growth response to different nutrient conditions in the field experiment. The grey columns represent resupplying nutrients on the day. The error bars correspond to the standard deviation of triplicates.
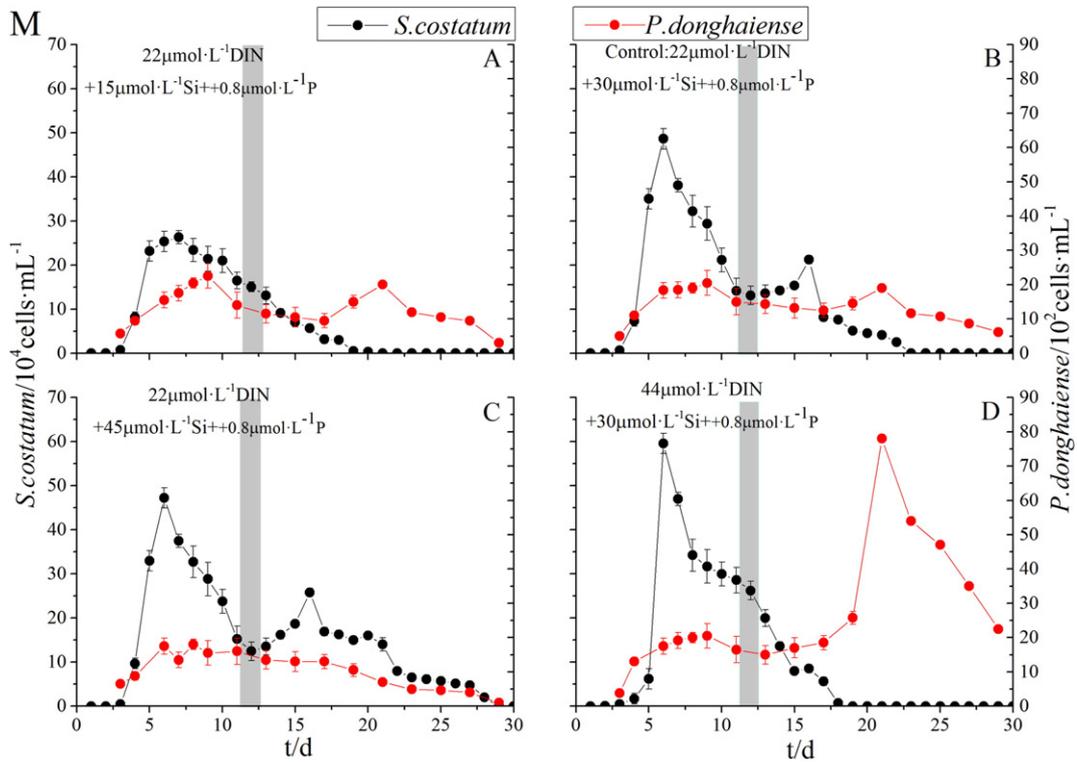
Fig. 3. The changes of diatom *S. costatum* (black spot) and *P. donghaiense* (red spot) growth response to different nutrient conditions in the indoors experiment (group M). The grey columns represent resupplying nutrients on the day. The error bars correspond to the standard deviation of triplicates.

was constant, addition of DIN and P did not heighten the dominance of *S. costatum* ($p > 0.1$, Figs. 5, 6 and 7). After resupplying nutrients, D_t tended to be less than zero, which indicated that *P. donghaiense* started to dominate the phytoplankton communities. However, in the culture with 45 μmol·L^{-1} Si of the experimental series M, D_t remained higher than zero throughout culturing, which indicated that *S. costatum* still dominated phytoplankton communities in the culture (Fig. 6). In other experimental cultures, min-D_t was less than zero. Moreover, high DIN concentration would double dominance degree of *P.*

donghaiense relative to control ($p < 0.01$, Figs. 6B, D, 7A and B), especially for cultures with high DIN and lower phosphate concentration (Fig. 7B).

3.3. Evolution of nutrients concentration and structure

Changes in nutrient structure were shown in Figs. 5, 6 and 7. N/P did similar temporal responses in all cultures. Initially, N/P ratio peaked on days 4–6 in field and on days 2–5 in indoor experiments, and N/P ratio increased again after resupplying nutrients, especially for indoors
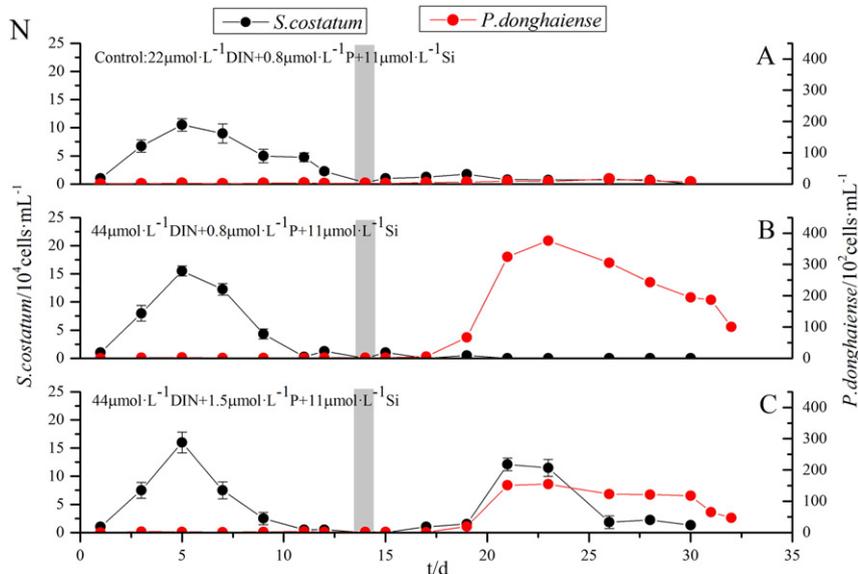
Fig. 4. The changes of diatom *S. costatum* (black spot) and *P. donghaiense* (red spot) growth response to different nutrient conditions in the indoors experiment (group N). The grey columns represent resupplying nutrients on the day. The error bars correspond to the standard deviation of triplicates.
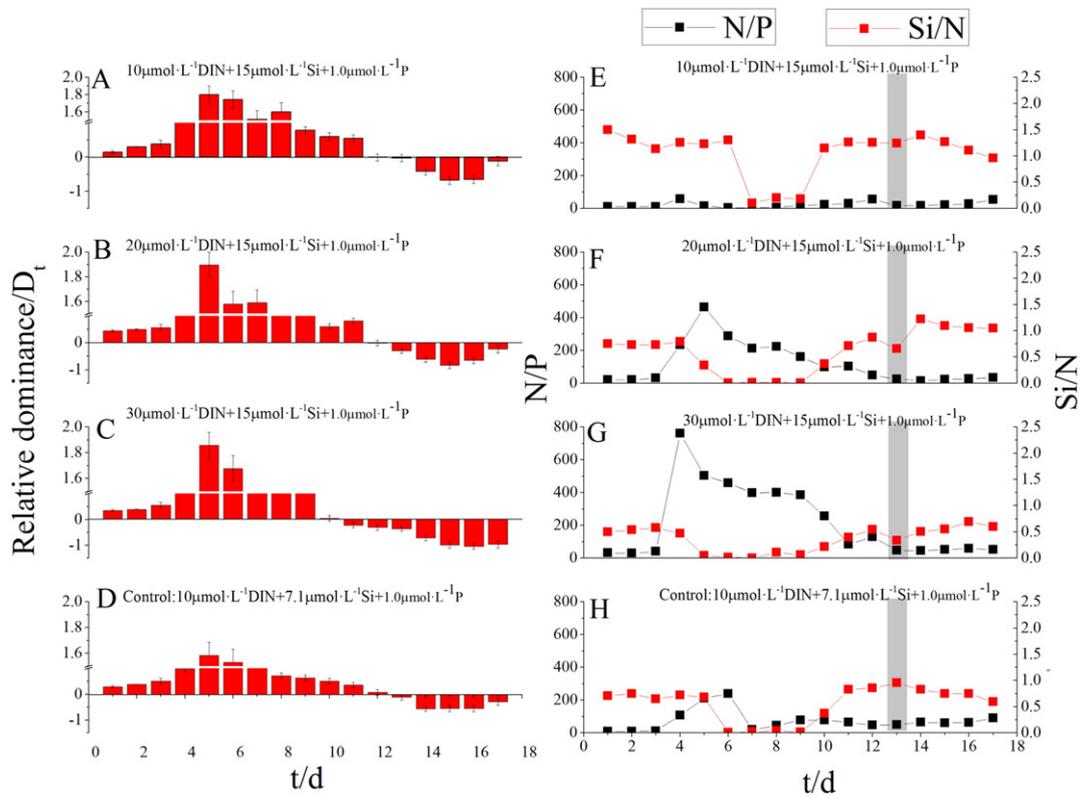
Fig. 5. The variation of relative dominance parameter D_t (A, B, C, D) and nutrients structure (E, F, G, H) in the Field experiment. The grey columns represent resupplying nutrients on the day. The error bars correspond to the standard deviation of triplicates.
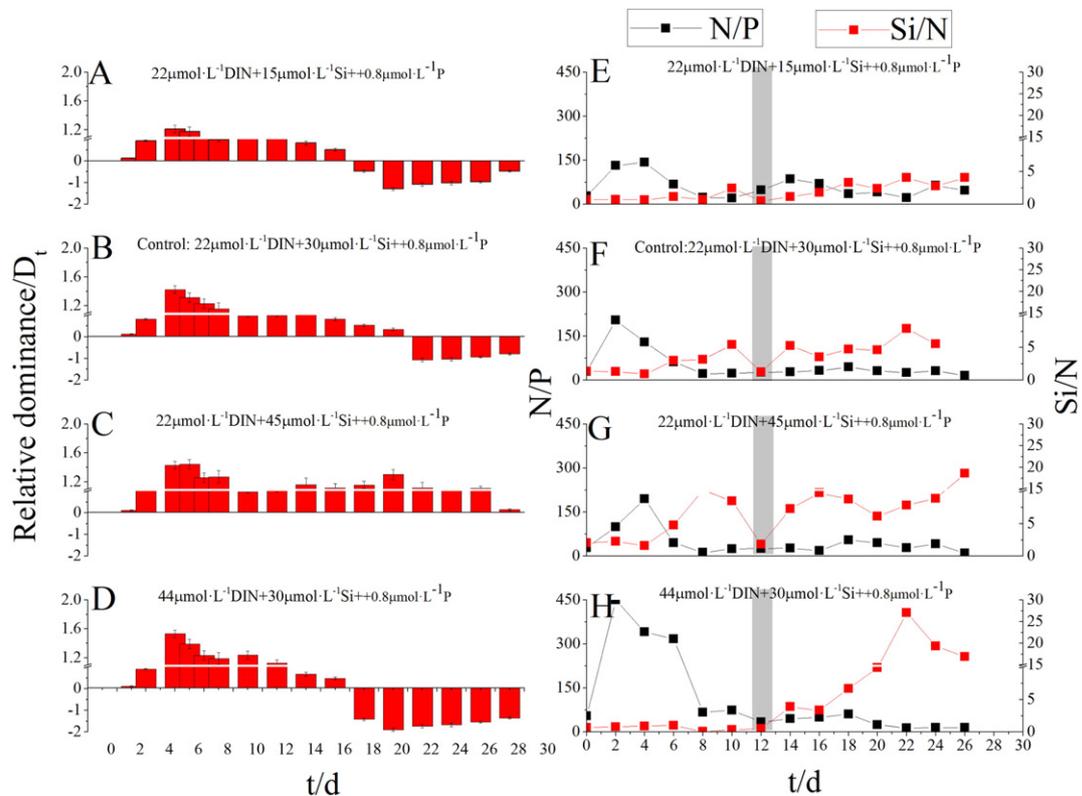
Fig. 6. The variation of relative dominance parameter D_t (A, B, C, D) and nutrients structure (E, F, G, H) in indoors experiments (group M). The grey columns represent resupplying nutrients on the day. The error bars correspond to the standard deviation of triplicates.
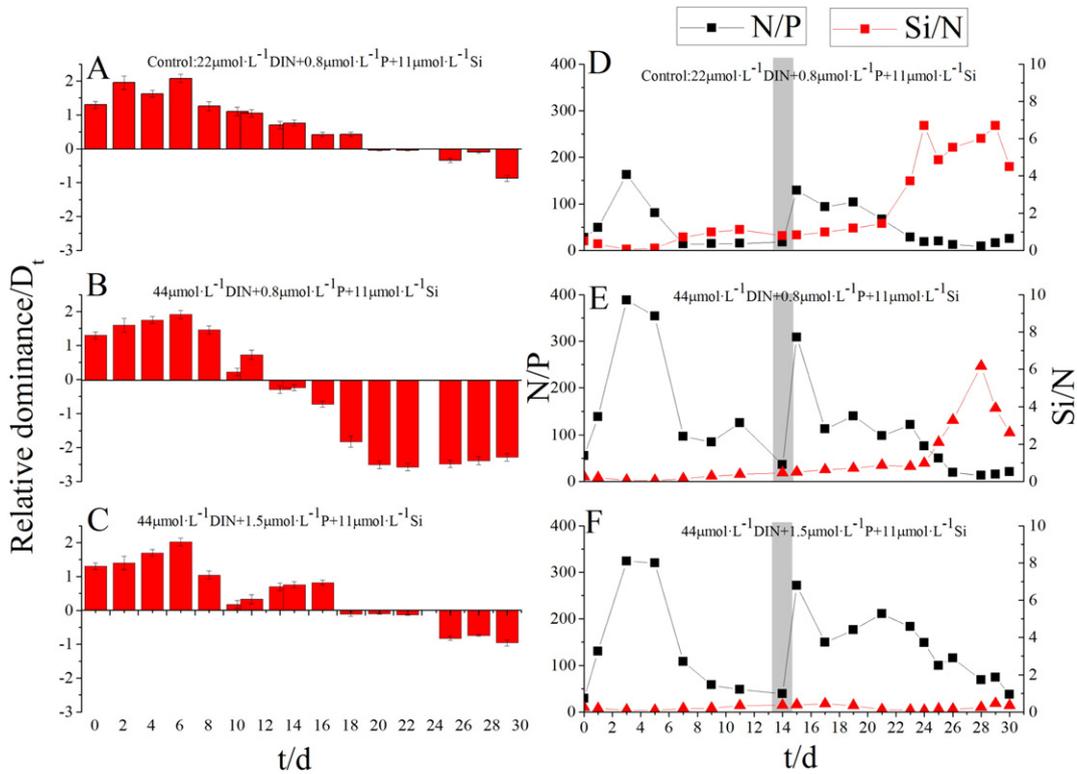
Fig. 7. The variation of relative dominance parameter D_t (A, B, C) and nutrients structure (D, E, F) in indoors experiments (group N). The grey columns represent resupplying nutrients on the day. The error bars correspond to the standard deviation of triplicates.

experiment, and then subsequently decreased. Distinctly, Si/N ratio reached minimum on days 6–9 except in the culture with high initial Si/N (Si/N > 1, Fig. 6F and G), and then started to increase until culturing ended. In field culture A3 and indoor culture M4, Si/N had a significant relativity with relative dominance D_t when the succession of *S. costatum* to *P. donghaiense* happened (Fig. 8, R^2 > 0.8), which indicated nutrient structure variations agreed with phytoplankton community composition changes.

Maximum nutrient uptake content and utility rates were influenced by nutrient ratios and nutrient concentration, which were listed in Table 1. P utility rate was highest in all experiments throughout culturing. DIN and Si utility rates were relatively complex. In the field experiment, the initial utility rate of Si was relatively higher, nearly 100% due to the low

concentration of Si, whereas utility rate and uptake content of Si were lower after resupplied nutrients. DIN availability initially accumulated until the lower concentration indicated a higher utility rate, and resupplying nutrients still stimulated DIN uptake except in control (Table 1). In the indoor experiments, initial utility rate of Si in the experimental cultures was similar to the field experiment except the M3 culture which had higher Si/N (Si/N > 2) because Si utility rate was limited by lower DIN concentration. After resupplying of nutrients, Si was largely assimilated only in the M3 culture with higher Si/N and N3 culture with higher P concentration. During the whole experiment, DIN utility rate was higher, whereas low Si/N (Si/N < 1, M4&N2) reduced DIN utility rate. Moreover, DIN uptake content was different in the later stage because of different DIN concentrations in the media.
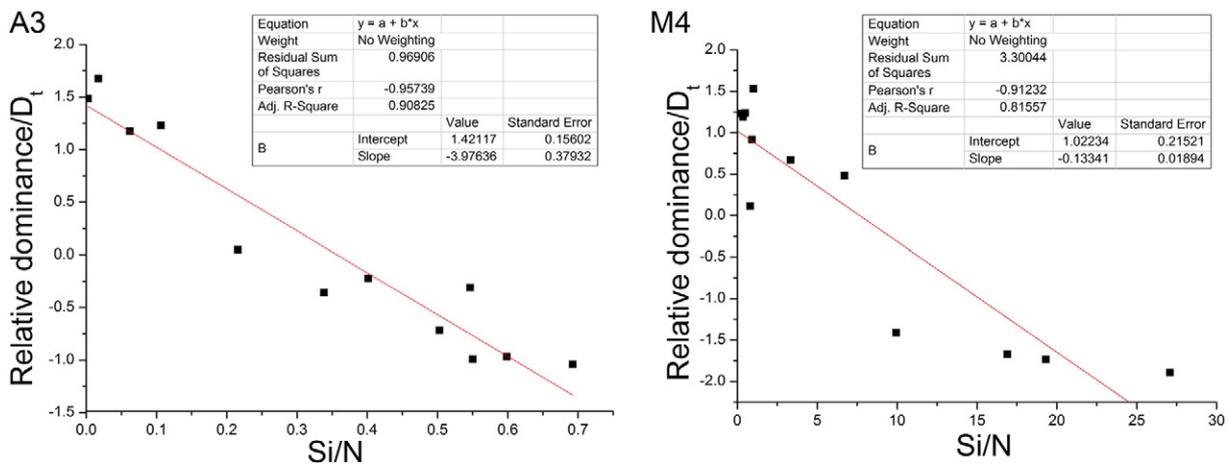
Fig. 8. The relationship between Si/N and relative dominance D_t when succession from *S. costatum* to *P. donghaiense* happened in culture A3 and M4.

Table 1
Maximum nutrients uptake content per volume (N_a and N_b represents maximum nutrients uptake content per volume in initial stage and late stage respectively) and maximum utility rate (U_a and U_b respectively represents maximum utility rate in initial stage and late stage).

No.	DIN				P				Si			
	N_a	U_a	N_b	U_b	N_a	U_a	N_b	U_b	N_a	U_a	N_b	U_b
Field experiment												
A1	9.90	99%	2.32	17.3%	0.95	96.9%	0.69	86.2%	14.87	99.1%	1.17	7.0%
A2	17.12	85.6%	10.1	43.5%	0.91	98.9%	0.75	84.1%	15	100%	0.38	2.5%
A3	20.83	69.4%	16.47	48.8%	0.92	97.9%	0.56	77.8%	14.84	98.9%	0.38	1.2%
Control	7.60	76.0%	–	–	0.98	99.0%	–	–	7.08	99.7%	–	–
Indoors experiment M												
M1	18.90	85.9%	13.7	80.1%	0.66	82.8%	0.64	86.8%	12.85	85.7%	–	–
M2	18.21	82.8%	16.11	81.0%	0.59	77.5%	0.66	88.6%	18.05	60.1%	8.66	34.7%
M3	19.92	90.6%	17.21	85.7%	0.61	77.8%	0.73	93.4%	17.89	39.8%	17.27	47.0%
M4	34.56	78.5%	23.93	87.9%	0.67	82.6%	0.74	93.2%	29.43	98.1%	–	–
Indoors experiment N												
N1	20.162	91.6%	12.75	89.3%	0.664	83%	0.684	84.5%	10.296	93.6%	1.551	13.8%
N2	32.213	73.2%	24.10	90.0%	0.678	84.8%	0.608	80.9%	9.802	89.1%	3.03	24.5%
N3	36.231	82.3%	24.13	78.3%	1.428	95.2%	0.683	87.1%	9.856	89.6%	9.512	86.4%

4. Discussion

4.1. Changes of nutrient and phytoplankton community in the ECS during the past 60 years

Nutrient concentration and structure in the ECS have changed dramatically in the past 60 years. DIN average concentration has abruptly increased from 5 $\mu mol \cdot L^{-1}$ in 1959 to 25 $\mu mol \cdot L^{-1}$ in 2010 owing to the large input of nitrate to the ECS from the Changjiang River estuary (Cheng and Li, 1992; Li et al., 2013). PO_4-P concentration increased from 1959 to 2000 and remained at about 0.8 $\mu mol \cdot L^{-1}$ since 2000 (Chen et al., 2004; Wang, 2006). By contrast, construction of the Three Gorges Dam reduced SiO_3-Si flux from 245×10^4 $t \cdot y^{-1}$ to 75 $t \cdot y^{-1}$ and mean SiO_3-Si concentration decreased from 35 $\mu mol \cdot L^{-1}$ in 1959 to 18 $\mu mol \cdot L^{-1}$ in 2010 (Li et al., 2007; Li et al., 2014). Correspondingly, nutrient structure of ECS also changed significantly in recent decades. Previous investigations showed a sharp increase in N/P ratio from 40 in the 1960s to 200 in 2010 and decrease in Si/N ratio from 2.5 in the 1990s to 1.0 in 2007 (Li et al., 2013). Field investigations showed that diatom abundance decreased by 26% and dinoflagellate abundance increased by 240% (Lin et al., 2008, He and Sun, 2009). Similarly, following the considerable annual variation in nutrients in ECS and adjacent areas, the seasonal variations of DIN, PO_4-P and SiO_3-Si during the past fifty years also changed significantly. Nutrient levels and structure fluctuated following the seasonal succession from diatom *S. costatum* to dinoflagellate *P. donghaiense*. Furthermore, statistical analysis indicated that nutrient amount might be an important factor for the shift of phytoplankton community in the ECS (Zhang et al., 2012).

4.2. Nutrient effects on phytoplankton biomass and community composition

Growth and relative abundance of *S. costatum* and *P. donghaiense* are influenced by nutrient concentration and structure. In the initial stage of culturing, *S. costatum* grew rapidly to dominate the phytoplankton communities, which was similar to the laboratory experiments by Li and Wang (2012). This was due to *S. costatum* having a stronger capacity for assimilating nutrients in high nutrient concentration compared with *P. donghaiense* and became dominant algae (DeMaster et al., 1995; Wang et al., 2006), which was demonstrated by a larger nutrient uptake in the initial stage of all experiments (Table 1). Si supply did not yield significant effects on the growth of *S. costatum* when Si/N was higher than 1. However, low Si/N due to decrease of Si concentration significantly restricted the growth of *S. costatum* and excessive DIN in the culture with lower Si/N (Si/N < 1, lower than Redfield ratio of 1) did not stimulate its growth. Therefore, Si concentration is still the decisive factor in *S. costatum* maxi-biomass, which is in line with earlier findings (Schelske et al., 1983). In most areas of ECS, Si/N was higher than 1 before 2000, therefore, the decrease in Si concentration did not influence the growth of *S. costatum*. By contrast, DIN addition yielded positive effects on the biomass of *S. costatum* when Si/N was higher than 1. Therefore, increasing DIN due to the large DIN fluxes of terrestrial discharge could be responsible for the increased eruption of *S. costatum* in ECS since 2000, wherein Si concentration remained at approximately 18 $\mu mol \cdot L^{-1}$ and Si/N was still higher than 1. Interestingly, addition of DIN did not improve the relative dominance of *S. costatum* in the cultures with a high Si/N and adding Si would enhance relative dominance of *S. costatum* when Si/N <1. Consequently, large input of DIN only stimulated the growth of *S. costatum* but not the abundance and low Si concentrations due to Three Gorges Dam determine the relative abundance of *S. costatum* in the ECS.

Excessive DIN would stimulate the later growth and relative abundance of *P. donghaiense* with a Si/N <1 in the media. However, high Si/N (Si/N > 1), especially with higher Si concentration, would keep dominant degrees of *S. costatum* and prevent the eruption of *P. donghaiense*. Currently, addition of higher P concentration reduced the relative dominance of *P. donghaiense* and facilitated a secondary eruption of *S. costatum*. DIN is superfluous and its maxi-concentration increased to 40 $\mu mol \cdot L^{-1}$ and P remained at approximately 0.8 $\mu mol \cdot L^{-1}$ in many areas of ECS since 2000 (Cao and Wang, 2012), therefore, excessive DIN stimulated the eruption of *P. donghaiense*. In the eutrophic environment of ECS, nutrient concentration and structure vary along with shifts of phytoplankton community. N/P ratio would rapidly peak due to the quick and sufficient assimilation of P by *S. costatum*, and then N/P would decrease as *P. donghaiense* dissipates (Zhang et al., 2012). Distinctly, Si/N initially decreased to its lowest value due to the continuous consumption of Si by *S. costatum*, and subsequently increased until *P. donghaiense* dissipated because the growth of *P. donghaiense* assimilated large amounts of DIN and decomposition of *S. costatum* released abundant Si (Li et al., 2014). In the experiment, nutrient structure change was similar to the field environment when phytoplankton communities shift. Moreover, high N/P and low Si/N existed upon succession of *S. costatum* to *P. donghaiense*. The results of the present study corroborated field investigations and suggested that low Si/N and high N/P induced the succession of *S. costatum* to *P. donghaiense*. Moreover, the high utility rate of DIN in later stage indicated that lower DIN concentration limited the growth and accelerated the decline of *P. donghaiense*. Field investigations also reported that *P. donghaiense* declined as DIN

concentration-is limited (Cao and Wang, 2012). However, the reason for *S. costatum* dissipation is still unclear. Low P has been asserted to favor *P. donghaiense* and limit *S. costatum* because of the different affinities of P (Wang and Wangm, 2013), but Du et al. indicated lower Si concentration accelerated the dissipation of *S. costatum* (Du et al., 2014). In this study, the addition of Si or P both kept dominant degrees of *S. costatum* and prevented the eruption of *P. donghaiense.* These findings broadened previous studies and strengthened the theory of interspecies competition. However, more studies are needed to further explore the limiting factors of *S. costatum* growth.

4.3. Implication for microalgal succession and nutrient management

Dinoflagellate and diatom had different adaptive strategies to nutrient conditions, both of which gave rise to competing for resources and succession driven by changes in nutrient concentration and structure (Glibert, 2016; Glibert et al., 2016). The present study demonstrated the clear relationship between large DIN flux and dominance of dinoflagellate in the ECS. Although the research objective of dinoflagellate, *P. donghaiense*, is not toxic, it also has a longer duration and broader influential area with significant negative impact on coastal economy and human health relative to *S. costatum* (Li and Wang, 2012). Moreover, toxic dinoflagellates, such as *Karenia mikimotoi*, erupted in ECS caused by a large DIN supply (Lu and Huang, 2007). Numerous methods such as controlled nutrient input flux and ecological remediation, have been applied to restore community structure. Ecological remediation technology, a new and cost-effective technology, can contribute to the restoration of diatoms and community structures (Xu et al., 2014), but the technology is mainly applied in eutrophic lakes that could be easily controlled. In addition, the mechanism of the technology is complicated and immature (Pu et al., 2012), therefore studies need to be done to amend and improve this technology. DIN concentration has continuously increased due to anthropogenic activities in ECS, which still change the relative abundance of diatoms, therefore, restoration of diatom populations is not sufficient to stabilize the community structure. Regulating the DIN supply in ECS is more effective and critical for reducing dinoflagellate blooms and protecting marine ecosystems. Furthermore, because of the global increasing trend of DIN relative to P into the coastal sea, N/P ratio exceeds the Redfield ratio (Childers et al., 2011; Glibert et al., 2013, 2014), which is responsible for the high frequency of harmful algal blooms in coastal areas worldwide (Heisler et al., 2008; Shipe et al., 2008; Selina et al., 2014). As programs were developed to reduce the input of Si, N management should be paid great attention by international managers to reduce the dinoflagellate biomass and toxicity. Briefly, the results derived from the field and lab experiments were consistent with the hypothesis that the change of N/P and Si/N would lead to succession between diatom (*S. costatum*) and dinoflagellate (*P. donghaiense*). Therefore, optimum management practices should be developed to mediate eutrophication along the coastal areas.

5. Conclusions

This study focused on the effects of nutrient structures on the competition and succession between diatom (*S. costatum*) and dinoflagellate (*P. donghaiense*) in ECS. In general, large spatial and temporal variabilities in the relative abundances of diatom and dinoflagellate were observed in the given coastal areas and given time. This funding suggested that their relative abundances were associated with nutrient changes. The parameter D_t would conveniently describe the relative abundance of the two algae. The relationship between low Si concentration and low relative abundance of *S. costatum* indicated that low Si concentration caused by the construction of the Three Gorges Dam is an important factor that inhibits the growth of *S. costatum*. This

phenomenon in turn benefits *P. donghaiense*. High N/Si and N/P induced by excessive DIN likely enhanced the relative abundance of *P. donghaiense*. Therefore, a large DIN flux caused by anthropogenic activities stimulated *P. donghaiense* bloom. Hence, relevant programs should be proposed to manage the input flux of nitrate to inhibit harmful dinoflagellate blooms in ECS.

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