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Adding nitrate and phosphate separately or together in the Central Indian Ocean: a nutrient enrichment experiment

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Abstract

Nutrient enrichment experiments were carried out in the Central Indian Ocean during the Chinese First Around-the world Research Cruise, adding nitrate, phosphate, or a mixture of both of them to surface seawater. The concentration of nitrate, nitrite, ammonia, and phosphate were analyzed spectrophotometrically, the chlorophyll-*a* concentration with fluorescence analysis, and the temperature variation during the experiment recorded. Addition of nitrate resulted in rapid growth of phytoplankton concomitant with depletion of nitrate in the water samples. No apparent variation occurred in chlorophyll-*a* concentration when phosphate was added. Combining nitrate and phosphate proved to be best to promote phytoplankton bloom, and nitrate was depleted prior to phosphate. After nitrate was consumed, a substantial amount of phytoplankton survived on the supplied phosphate. No correlation was found between the nitrate to phosphate ratio and chlorophyll-*a* or phytoplankton growth rate. We also found no correlation between water temperature and chlorophyll-*a* or phytoplankton growth rate. We conclude that neither nitrate to phosphate ratio nor water temperature control the growth of phytoplankton.

1 Introduction

A wide variety of nutrients are essential for phytoplankton growth in the oceans, including macronutrients such as nitrogen, phosphorus and silicon, and micronutrients such as iron and zinc. Nutrients limitation of phytoplankton has been reported extensively in different sea areas. Among those nutrients, nitrogen and phosphorus play a particularly important role in limiting biological productivity as evidenced by their often near complete exhaustion in surface waters (Gruber, 2004). The question arises if nitrate or phosphate or both are the primary nutrients controlling phytoplankton production? Different answers have been given in nutrient enrichment experiments depending on the sea in which they were carried out. Nitrogen is the most important nutrient in limiting

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phytoplankton growth in the oligotrophic tropical Pacific Ocean (Thomas, 1966, 1967, 1969, 1970), South Pacific subtropical region (Dufour et al., 1999; Dufour and Berland, 1999), South China Sea (Chen et al., 2004), Kaneohe Bay of Hawaii (Larned, 1998) and Cape Bolinao of NW Philippines (Terrados, 1999). However, phosphorus limited growth occurs in the northwest Mediterraneann (Thingstad et al., 1998), the East China Sea (Wong et al., 1998), and Bohai Sea (Zou and Zhang, 2001). Nitrogen and phosphorus may all well limit phytoplankton production in Daya Bay (Wang et al., 2007; Zhu et al., 2008), Taiwan Strait (Wang et al., 2008), and specific areas in the Yellow and East China Sea (Liu et al., 2004).

Nitrogen or phosphorus control on phytoplankton growth varies per sea. In the Indian Ocean, there has been some research on the nutrient limitation of phytoplankton. Phytoplankton growth could be stimulated by the addition of either NH_4^+ or NO_3^- and co-limited by both Fe and macronutrients in the central area of the northwestern Indian Ocean (Takeda et al., 1995). NH_4^+ is also the major nitrogenous nutrient used by phytoplankton in the western Indian Ocean (Mengesha et al., 1999). To investigate phytoplankton nutrient control in the Central Indian Ocean, we carried out a nutrient enrichment experiment by adding nitrate, phosphate, or both to surface sea water. Water temperature, the concentration of nitrate, nitrite, ammonia, and phosphate, and chlorophyll-*a* concentration were monitored during the experiment. The influence of water temperature, nutrient depletion, and N:P on phytoplankton growth was presented.

2 Materials and methods

Our nutrient enrichment experiment was conducted in the central Indian Ocean during the Chinese First Around-the world Research Cruise in December 2005. The cruise ran along the Mid-Indian ridge to explore hydrothermal vents. About 12 full-depth CTD and 12 multi-sampler stations were completed in the Indian Ocean. Data were collected both by continuously recording instruments such as CTD and deep-towed devices, and

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individual sample analysis for nutrient, chlorophyll-*a*, CH_4, biodiversity, etc. The primary productivity was influenced by the prevailing northeast monsoon at that time. Surface seawater for nutrient enrichment experiment was collected from 50.17° E, 37.81° S.

The experimental facility set on the bow consists of four 500-L barrels each with a diameter of ca. 100 cm, a set of cooler and a temperature controller (Tang et al., 2009). The side wall of acrylic barrels is transparent, ensuring enough light into the barrels. Cooling water from the cooler could cycle within the rubber tubes, which are circled through each barrel. Thus, The cooler and temperature controller could adjust the experimental temperature close to that of the actual surface seawater.

All sampling and incubator equipment was washed three times with surface seawater beforehand. Next surface seawater (50.17° E, 37.81° S) was collected with a vacuum pump in four acrylic barrels (B1, B2, B3, B4). The nutrient concentrations of our surface seawater samples are shown in Table 1. Seawater in B1 was used as background without nutrient addition. Nitrate, phosphate or mixtures of them were added separately into the other three barrels (see in Table 2) as inorganic salts: KNO_3, $FeSO_4{\cdot}7H_2O$, and KH_2PO_4. Next the sea water in the barrels was stirred with a glass rod and left to stay for 17 d. The sea water was sampled every 12 or 24 h to analyze for nutrients spectrophotometrically, and for chlorophyll-*a* with a fluorescence method. The temperature of each of the barrels was measured when sampling.

3 Results and discussion

3.1 Phytoplankton growth

Chlorophyll-*a* concentration is an important parameter in reflecting phytoplankton growth. The highest average concentration of chlorophyll-*a* (Chl_{ave}) was present in B4 with the addition of nitrate and phosphate, whereas Chl_{ave} in the background barrel (B1) approximated that of B3 with the addition of phosphate, with a value below of that in B2 and B4. The variation of chlorophyll-*a* concentration is shown in Fig. 1a. The fist

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maximum of chlorophyll-*a* concentration as a function of time is defined as the blooming spot of phytoplankton (BSP). BSP occurred on the 8th day of the experiment for B2 and B4 (see in Fig. 1a). The increase of chlorophyll-*a* concentration at BSP (Chl_{BSP}) in B4 was the most pronounced, being 2.56 $mg m^{-3}$ higher than the initial value (Chl_{ini}). Chl_{BSP} in *P*-addition barrel (B2) increased 0.92 $mg m^{-3}$. However, no apparent increase in chlorophyll-*a* concentration appeared in B1 and B3, i.e. the background barrel and the one to which only phosphate was added. Clearly, phosphate addition has little influence on phytoplankton growth. Nitrate addition strongly promoted phytoplankton bloom, which was also proved by the nutrient enrichment experiment in the northwestern Indian Ocean (Takeda et al., 1995). The pronounced maximum in B4 indicates that addition of nitrate together with phosphate proved to be best in promoting phytoplankton growth. In addition, the average concentration of chlorophyll-*a* after BSP (Chl_{aBSP}) was much higher than Chl_{ini} in both B2 and B4 (see in Fig. 1a and Table 3), indicating that a substantial concentration of phytoplankton managed to survive. The abundance of phytoplankton after BSP in B4 was higher than that in B2.

The phytoplankton growth rate (R) as a function of time is calculated as follows:

$$R = \ln(Chl_t / Chl_{ini})/t \quad (1)$$

where t is the incubation time and Chl_t is the concentration of chlorophyll-*a* at time t.

R in the four barrels is shown in Fig. 1b and Table 3. The average value of R (R_{ave}) was highest in B4. R_{ave} in B1 and B3 was similar but much lower than that in B2 and B4. The value of R in the four barrels increased rapidly and similarly at the beginning of the experiment, but decreased rapidly after the initial pulse in the background barrel (B1) and P-addition barrel (B3). Maxima in R appeared at BSP in B2 and B4, corresponding with a maximum in chlorophyll-*a* concentration. These results suggest that adding nitrate results in rapid growth of phytoplankton, whereas adding phosphate did not. R_{BSP} in B4 was 0.12 d^{-1} higher than that in B2. We conclude that adding nitrate and phosphate enhanced phytoplankton growth relative to adding nitrate only.

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3.2 The influence of water temperature on phytoplankton growth

The average water temperature was ca. 21.5°C during our experiment. The temperature variation is shown in Fig. 2. The temperature increased from 18°C on the 4th day to 23.5°C on the10th day: the most pronounced temperature trend in our experiment. Later on the water temperature decreased from 23°C to 22°C with an average of 22.5°C. The most rapid increase in chlorophyll-*a* concentration and in *R* in B2 and B4 all occurred from the 6th to 8th day, which coincided with the period in which temperature increased. This indicates that increasing water temperature has some effect on phytoplankton growth. Previous studies (Eppley, 1972; Goldman et al., 1974; Yoder, 1979) have proved that temperature plays an important role in the growth of diatoms. However, the community sensitivity to temperature may be far smaller than the species sensitivity because of the adaptation of different organisms to a specific temperature range (Sarmiento and Gruber, 2006). As shown in Table 4, there was no significant correlation between water temperature and chlorophyll-*a* concentration in the four barrels. Water temperature was negatively correlated with *R* in B1, B2, and B3, whereas no correlation showed up in B4. We conclude that, at least in our experiment, temperature does not dominate the growth of phytoplankton community.

3.3 The influence of nutrient addition to phytoplankton growth

The variation in concentration of nitrate ($[NO_3]$) and phosphate ($[PO_4]$) in the barrels is shown in Fig. 3 and Table 3. $[NO_3]$ in B1 (no addition of nutrient) and B3 (P addition) was low and show no obvious change trends, whereas that in B2 (N addition) and B4 (N and P addition) went in general down. Within the first 9 days, $[NO_3]$ was reduced from 11.55 μM to 6.86 μM in B2 and from 12.63 μM to 0.35 μM in B4, concomitant with an increase in chlorophyll-*a* concentration. Especially from the 7th to 9th day in our experiment, the drop in $[NO_3]$ from 8.46 μM to 0.35 μM in B4 occurred synchronously with phytoplankton bloom. $[NO_3]$ after BSP remained high in B2 with an average of 6.04 μM, supplying apparently the essential nutrient for the survival of sub-

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stantial amounts of phytoplankton. We conclude from these results that nitrate plays an important role in phytoplankton growth.

The average of $[PO_4]$ ($[PO_4]_{ave}$) in B1 was similar to that in B2, and they all showed a flat concentration profile with time. No obvious depletion of phosphate in B3 occurred with low nitrate and low chlorophyll-*a* levels (see Table 3). We infer that adding only phosphate does not enhance growth of phytoplankton. In B4 with the addition of nitrate and phosphate, $[PO_4]$ went down after the 6th day. It went down to 2.06 μM on the 9th day during phytoplankton bloom concomitant with a rapid decrease of $[NO_3]$ to 0.35 μM. This proved that nitrate is used up prior to phosphate. $[PO_4]$ continued to decrease subsequently to 1.36 μM at the end of the experiment. After BSP, a substantial amount of phytoplankton was present in B4 (see in Fig. 1a) whereas $[NO_3]$ was close to that in B1 (see in Fig. 3a). Our inference is that phytoplankton continued to live on phosphate.

R in all four barrels increased rapidly during the first day of our experiment (see in Fig. 1b). Neither $[NO_3]$ nor $[PO_4]$ showed significant and uniform variation (see in Fig. 3). This increase of *R* has little relationship with the variation of nutrient concentration in the experiment, as is also proved by the poor correlations ($P>0.05$) between *R* and $[NO_3]$ or $[PO_4]$. When *R* in B4 went up from 0.28 d^{-1} on the 6th to 0.46 d^{-1} on the 8th, $[NO_3]$ decreased from 8.69 μM to 3.74 μM, and $[PO_4]$ from 2.65 μM to 2.27 μM. In B2, *R* increased 0.07 d^{-1} from the 6th to 8th day with slight reduction of 0.31 μM in $[NO_3]$. We infer that the increase of *R* around BSP is mainly caused by phytoplankton bloom with the ingestion of nitrate, phosphate, or both.

3.4 The influence of N:P to phytoplankton growth

N:P represented the ratio of dissolved inorganic nitrogen (DIN: $NO_3+NO_2+NH_3$) to dissolved inorganic phosphorus (DIP: PO_4), shown in Fig. 4 and Table 3. Note the difference in scale for this ratio in Fig. 4. The average was highest in B2 and the lowest in B3. Previous studies (John and Flymn, 2000; Plinski and Jozwiak, 1999) have indicated that N:P ratio was an important factor to influence the growth of phytoplankton species. Because the optimal N:P promoting phytoplankton growth varied among

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different species, it is thought that N:P had much more impact on species than phytoplankton community. In B1 with low nitrate and low phosphate, N:P ranged from 3.61 to 12.24 without clear change trend, and no obvious phytoplankton bloom occurred. In B2 with high nitrate, a maximum in N:P appeared on the 8th day when phytoplankton was blooming. In B3 with high phosphate, the N:P trend line showed an obvious peak on the 7th day, while the chlorophyll-*a* concentration remained stable. In B4 with high nitrate and high phosphate, N:P reduced rapidly when phytoplankton was blooming. Thus, N:P in our experiment did not control the growth of phytoplankton community, as confirmed by the poor correlations between N:P and chlorophyll-*a* concentration or *R* (see in Table 4).

4 Conclusions

We conducted nutrient enrichment experiments by adding nitrate, phosphate or a mixture of them to surface seawater in the Central Indian Ocean. Temperature, chlorophyll-*a* concentration, and nutrient concentration were investigated. Several conclusions may be drawn from our experiment to wit:

i) The addition of nitrate stimulates phytoplankton bloom, but phosphate addition does not. Adding nitrate and phosphate together proved to be the best in promoting phytoplankton growth.

ii) With the addition of nitrate and phosphate, phytoplankton growth uses them in tandem. Nitrate is depleted prior to phosphate. After nitrate depletion, phytoplankton continues to grow using phosphate.

iii) Neither temperature nor N:P controls the growth of phytoplankton community.

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Table 1. Background value of experimental sea water in the Central Indian Ocean.

NO_3 (μM)	NH_4 (μM)	NO_2 (μM)	PO_4 (μM)	SiO_3 (μM)	Chlorophyll-*a* (mgm^{-3})
0.09	0.62	0.05	0.15	0.62	0.32

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Table 2. Nutrient addition in different barrels in the experiment.

Number of barrel	N addition (mol)	P addition (mol)
B1	*	*
B2	0.005	*
B3	*	0.0007
B4	0.005	0.0007

* represent no addition of N or P.

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Table 3. Summary of the concentration of chlorophyll-*a*, [NO_3], [PO_4], R, and N:P in the experiment. Chl_{ave}, $[NO_3]_{ave}$ and $[PO_4]_{ave}$ are the average concentration of chlorophyll-*a*, nitrate, phosphate; R_{ave} is the average rate of phytoplankton growth in the experiment; Chl_{ini} and $[NO_3]_{ini}$ are the initial concentration of chlorophyll-*a* and nitrate measured on the first day of the experiment; Chl_{BSP} and R_{BSP} are the chlorophyll-*a* concentration and R at BSP; Chl_{aBSP} is the average concentration of chlorophyll-*a* after BSP; $[NO_3]_{ter}$ is the concentration of nitrate at the end of the experiment; N:P is the ratio of DIN (NO_3+NO_2+NH_3) to DIP (PO_4).

Number of barrel	Chl_{ave} (mgm^{-3})	Chl_{ini} (mgm^{-3})	Chl_{BSP} (mgm^{-3})	Chl_{aBSP} (mgm^{-3})	R_{ave} (d^{-1})	R_{BSP} (d^{-1})	$[NO_3]_{ave}$ (μM)	$[NO_3]_{ini}$ (μM)	$[NO_3]_{ter}$ (μM)	$[PO_4]_{ave}$ (μM)	N:P
B1	0.12	0.06	0.10	0.15	0.09	0.06	0.12	0.08	0.15	0.15	6.83
B2	0.34	0.06	0.98	0.41	0.18	0.34	8.14	11.56	6.98	0.13	80.97
B3	0.11	0.07	0.10	0.12	0.06	0.05	0.07	0.08	0.09	2.18	0.47
B4	0.74	0.07	2.63	1.03	0.23	0.46	4.63	12.63	0.8	2.11	2.33

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Table 4. Result of correlation analysis between temperature and chl-*a*, temperature and *R*, N:P and chl-*a*, and N:P and *R*. *T* is water temperature; *C* is correlation coefficient; *P* is significance level; chl-*a* represents the concentration of chlorophyll-*a*.

Number of barrel	*T*–chl-a	*T*–*R*	N:P–chl-*a*	N:P–*R*
B1	P=0.493	C=−0.910; P=0.000	P=0.217	P=0.397
B2	P=0.265	C=−0.548; P=0.028	P=0.065	P=0.537
B3	P=0.519	C=−0.940; P=0.000	P=0.067	P=0.442
B4	P=0.090	P=0.668	P=0.063	P=0.129

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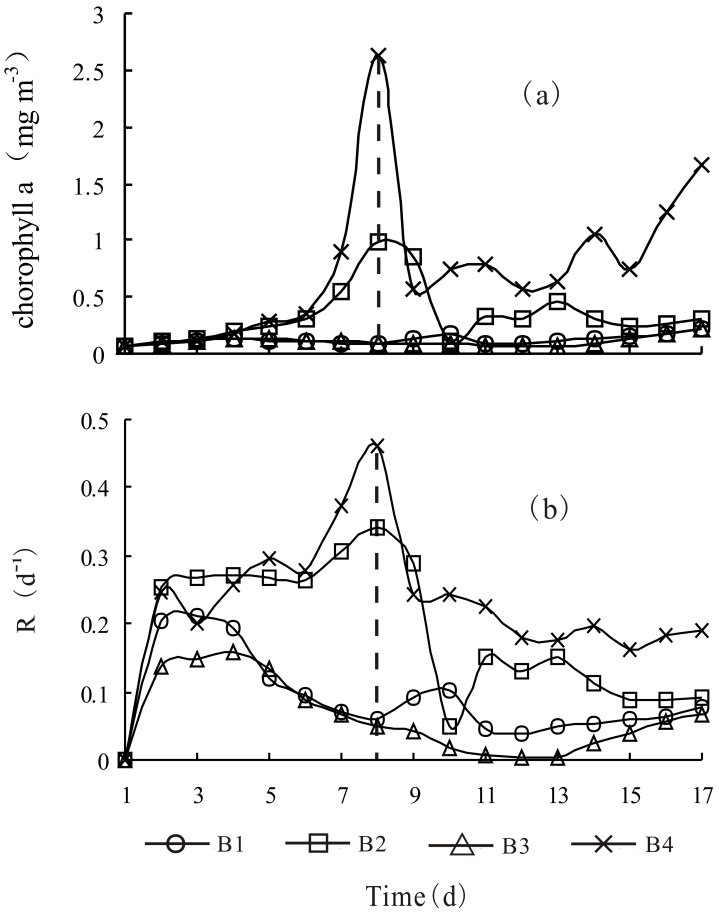

Fig. 1. Trends of chlorophyll-*a* and phytoplankton growth rate (*R*) in the four barrels during the experiment. The broken line marked the time when BSP appeared.

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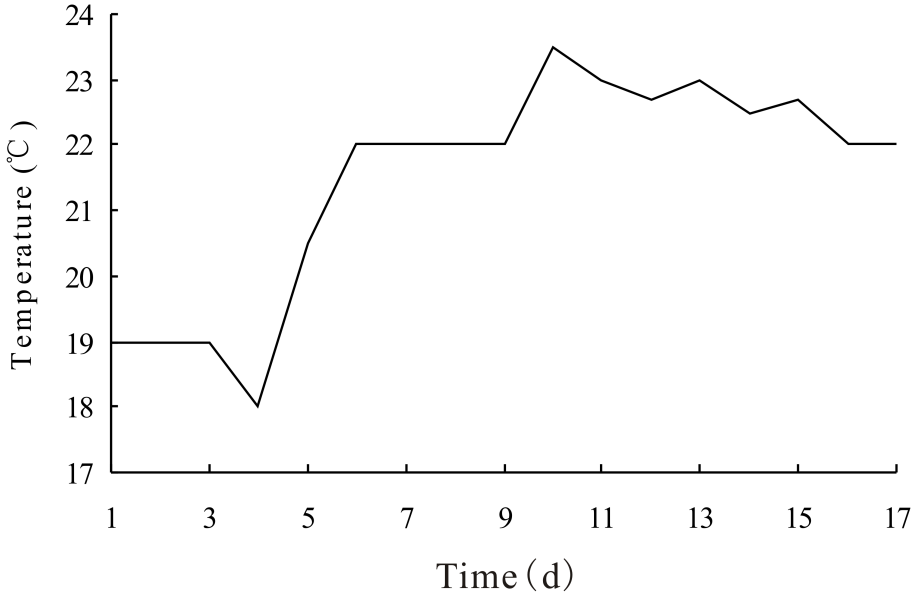

Fig. 2. Temperature variation in the experimental water.

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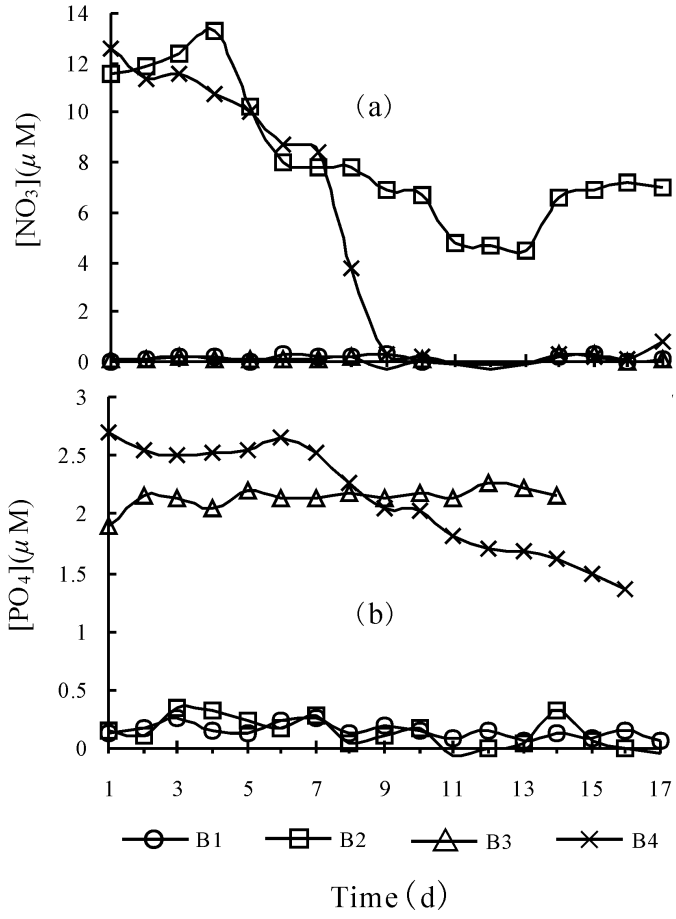

Fig. 3. The variation of $[NO_3]$ and $[PO_4]$ in experimental water.

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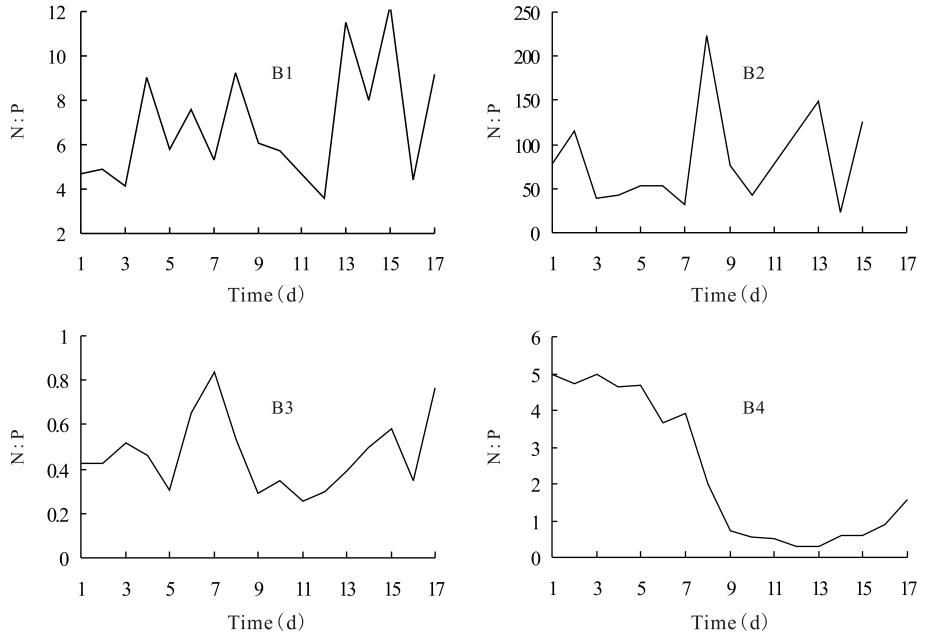

Fig. 4. The variation of nitrate phosphate ratio in the experimental water.

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