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Nitrogen modulates phytoplankton growth in spring in the South China Sea

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Abstract

The present study investigated the roles of nitrogen (N) in modulating phytoplankton growth in the South China Sea (SCS), an oligotrophic marginal sea in the western Pacific Ocean, by examining (1) the in situ nitrate and nitrite concentrations [$NO_3 + NO_2$]; (2) the [$NO_3 + NO_2$]/[soluble reactive phosphorus (SRP)] in the euphotic layer; (3) phytoplankton growth in response to nutrient enrichments; and (4) the spatial distribution of nitrate-based new production and primary production and the factors controlling their dynamics. N_2-fixation rates of the planktonic cyanobacteria, *Trichodesmium* sp. and the diatom-symbiont *Richelia intracellularis*, were estimated based on the abundance of the N_2-fixers and were compared to the nitrogen demands estimated based on the nitrate uptake rate (nitrate-based new production). The comparison evaluates the relative importance of two sources of new nitrogen, the atmosphere (N_2) vs. the deep waters (NO_3^-). The study was conducted on cruises in March 2000 and March 2001 when the northeastern monsoon was prevailing in the SCS. Both N and phosphorus (P) were scarce in the euphotic zone; surface [$NO_3 + NO_2$] was in the range of 9–309 nM and [SRP] ranged from 6 to 88 nM. Surface [$NO_3 + NO_2$] was negatively ($p < 0.01$) correlated with stratification index of water column. The ratios of [$NO_3 + NO_2$]/[SRP] were much smaller than the Redfield N/P Ratio of 16:1, suggesting a state of N-limitation. The water of the shallow shelf-slope was less stratified. Its surface nitrate concentration, chlorophyll *a* concentration, nitrate-based new production and total primary production were higher than those of the deep basin water. Simulated production experiments on the shelf and the basin revealed that nitrate-based new production ranged from 0.06 to 0.36 g C m^{-2} d^{-1} whereas primary production ranged from 0.12 to 0.63 g C m^{-2} d^{-1}. Both productions are positively ($p < 0.01$) correlated with the surface nitrate concentration. The results of the enrichment experiments showed that N alone limited phytoplankton growth. Enrichment with nitrate only or with nitrate plus phosphate resulted in a phytoplankton bloom as shown by the enhanced chlorophyll *a* concentration, chlorophyll *a*-specific primary and new productions. Similar to the control group (without nutrient addition), enrichment with only phosphate did not enhance algal growth. The nitrogen-fixing cyanobacteria, *Trichodesmium* sp. and *R. intracellularis*, were both scarcely distributed. Input to new production by N_2-fixation was at most 0.34 μg N m^{-3} h^{-1}, constituted less than 3% of the new production, even a generous estimate of the N_2-fixation rate was used in the estimation. This indicates that nitrate diffusing from the deep water was the main source of new nitrogen for phytoplankton growth in the SCS during the early spring. This finding is supported by the

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negative relationship ($p<0.01$) between the stratification index of water column and the surface [$NO_3 + NO_2$], and the positive relationship ($p<0.01$) between [$NO_3 + NO_2$] and the productivities. Our results show clearly that N limits phytoplankton growth in early spring in the SCS where N_2-fixation contributes little to new production in this oligotrophic system that iron deficiency is deemed unlikely.
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Keywords: New production; Primary production; *Trichodesmium*; South China Sea; Nutrient enrichment

1. Introduction

Nitrogen (N) is the most common element limiting phytoplankton growth in most marine ecosystems (Vitousek and Howarth, 1991). However, in some particular waters, such as the Mediterranean Sea (Thingstad and Rassoulzadegan, 1995; Thingstad et al., 1998), some estuarine and coastal waters of China (Harrison et al., 1990) and the Dutch coastal water (Cadée and Hegeman, 1993), phosphorus (P) limits phytoplankton production. Different scenarios have been proposed to infer the likelihood of the marine ecosystems to become P deficient or N deficient for phytoplankton growth. On one hand, P is more rapidly recycled than is N (Bishop et al., 1977; Martin et al., 1979). Consequently, P deficiency is less likely to occur than is N deficiency. Analysis of a geochemical budget, however, shows an opposite picture. Because N can be fixed from the atmospheric source (Vitousek and Howarth, 1991) and there is no atmospheric or other known alternative source for P, P is more likely to become short of supply than is N (Meybeck, 1982). Being the major pathway to bring the atmospheric N_2 into the organic food chains, N_2-fixation plays a significant role in modulating primary production and new production in marine ecosystems, especially in oligotrophic seas. The N_2-fixation process requires substantial quantities of iron (Rueter et al., 1990; Wu et al., 2000). The short supply of iron in most open oceans limits N_2-fixation and, thus, new N supply (Falkowski, 1997). These oceans may slowly lose N and become N-deficient because denitrification removes reactive nitrogen more rapidly than N_2-fixation imports it (Codispoti, 1995). This implicates that in an iron-rich marine ecosystem that favors N_2-fixation, an N-deficit is unlikely to occur so as to limit phytoplankton production.

The South China Sea (SCS) is an oligotrophic body of water, located in the subtropical and tropical western Pacific. Both N and P in its euphotic layer are usually below the detectable limits when measured by conventional methods (Gong et al., 1992). Unlike the low abundance of eolian iron in the eastern Pacific equatorial region, the atmospheric fluxes of iron in the western Pacific region are three-orders higher in magnitude (Barber and Chavez, 1991; Duce and Tindale, 1991). The setting of the SCS, thus, offers an excellent stage to test the aforementioned notion that nitrogen will not limit phytoplankton production in an ocean in which atmospheric flux of iron is high (Tyrrell, 1999).

The SCS is one of the world's largest marginal seas. It has an area of about 3.5×10^6 km^2, extending from the equator to 22°N (Fig. 1). In its northeastern region, a deep basin (maximum depth 5000 m) occupies almost a half of the surface area of the SCS (Fang et al., 1998; Shaw and Chao, 1994). The basin is surrounded by broad continental shelves less than 100 m in depth. The Luzon Strait located between Taiwan and Luzon is the principal deep channel (Fig. 1) allowing exchange with the Pacific Ocean. All other channels of the SCS are either shallow or narrow. The Kuroshio current intrudes the northern SCS through the Luzon Strait between October and February and transports westward its top 300 m of water along the SCS continental slope (Shaw and Chao, 1994). The SCS is within the East Asia monsoon region. The northeastern monsoon prevails in winter and spring, whereas the southwestern monsoon prevails in summer and autumn.

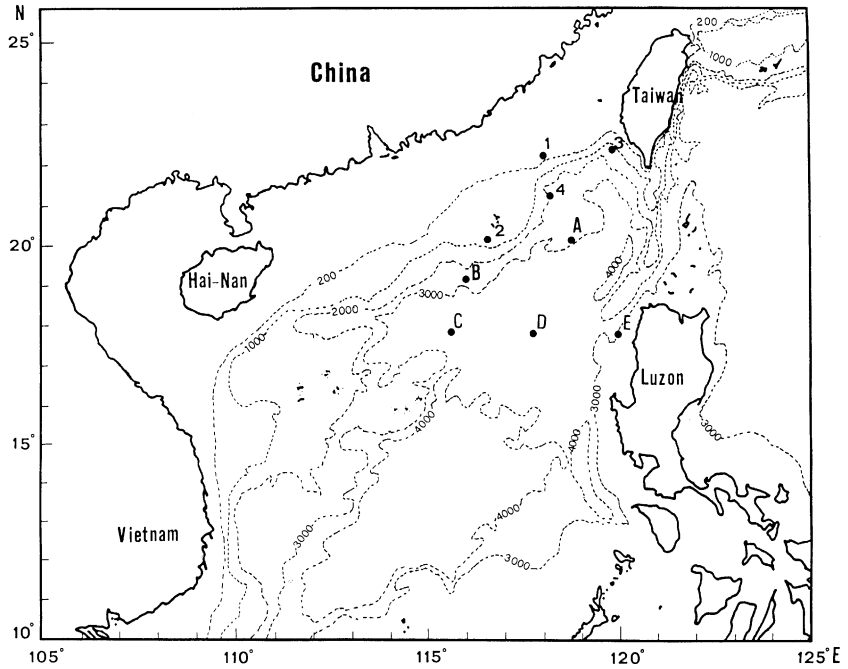


Fig. 1. Map of the South China Sea depicting the nine sampling stations.

This study studied the nitrogen status of the SCS in spring and its effects on phytoplankton growth by examining: (1) the relative abundance of nitrate and soluble reactive phosphorus (SRP) in the euphotic layer; (2) the responses of phytoplankton growth to nutrient enrichments; and (3) the spatial dynamics of nitrate-based new production and primary production and their possible controlling forcing. The relative importance of N_2-fixation by planktonic cyanobacteria to nitrogen demand from nitrate uptake (nitrate-based new production) was used to evaluate the role of N_2-fixation as a source of new N. To our knowledge, this is the first report describing the nitrogen dynamics in the SCS.

2. Materials and methods

Two cruises were conducted in the northern SCS (Fig. 1), on March 1–12, 2000 (RV/OR1, Cruise 575) and March 20–31, 2001 (RV/OR1, Cruise 606), respectively. Among the nine sampling stations studied, four stations (Stations 1, 2, 3 and 4) in the northwestern region were located over the shallow shelf or shelf slopes. The remaining five stations (Stations A, B, C, D and E) were located in the deep basin in the southeastern region. Phytoplankton productivities were measured at six stations (Stations 1,2, A, C, D and E). Nutrient enrichment experiments were carried out at both Stations 1 and C during the 2001 study and at Station C only during the 2000 study.

Water samples for nutrient analysis and for biochemical measurement were collected from the surface (100% light intensity) and from five light penetration depths (LPD) equivalent to 46%, 38%, 13%, 2% or 0.6% of the surface irradiance, respectively, using 20-1 Go-Flo bottles attached to a rosette multi-sampler fitted with CTD probes (Seabird SBE 9/11). Surface and subsurface irradiance were measured with a PAR sensor (OSP200L, Biospherical Inc., USA). The depth of the euphotic zone (Deu) is defined as the depth with 0.6% of the surface irradiance. Mixed layer depth was defined as the depth having a density (σ_θ) gradient less than 0.1 kg m^{-4}. The

stratification index (SI) is the average density difference (kg m^{-4}) between 150 m and the surface or between the bottom and the surface if water depth was less than 150 m.

The concentration of chlorophyll *a* was determined on acetone-extracted samples (Strickland and Parsons, 1972) using a fluorescence spectrophotometer (F3010, Hitachi Co., Tokyo, Japan). The ambient [NO_3] was measured on-board by the pink azo dye method using a flow injection analyzer (Pai and Riley, 1994). The method has a detection limit of 0.3 μM. When [NO_3] was below the detectable limit, the water sample was frozen and measured later in the laboratory. Nitrate and nitrite concentrations [$NO_3 + NO_2$] were determined for these samples by the chemiluminescent method (Garside, 1982). Soluble reactive phosphorus concentration [SRP] at the nanomolar level was determined by the modified-MAGIC method (Thomson-Bulldis and Karl, 1998) with the correction of arsenate interference. Silicate concentration was measured by the silicomolybdenum blue method (Pai et al., 1990).

Simulated shipboard experiments were conducted to estimate primary production of phytoplankton by measuring the uptake of $NaH^{13}CO_3$ (Hama et al., 1983). Uptake of $Na^{15}NO_3$ was conducted to estimate the nitrate-based new production (Dugdale and Wilkerson, 1986). Water was collected from six depths (the surface and five LPDs) and was immediately transferred into 2.3 l transparent polycarbonate bottles. The bottles were covered with neutral density screens to simulate the light intensity equivalent to that at respective LPD. Two light bottles and one dark bottle were assigned to each LPD. The bottles were incubated on deck for 3 h under natural light in transparent containers with flowing-through surface seawater. Before incubation, $NaH^{13}CO_3$ (99 at%, Isotec, OH, USA) was added to the primary production bottles to a final concentration of 0.19 mM; $Na^{15}NO_3$ was added to the nitrate-based new production bottles to a concentration equivalent to 10% of the ambient NO_3^- concentration [NO_3] measured on board. When [NO_3] was below the detectable limit, two final concentrations, 0.03 and 0.06 μM, of $Na^{15}NO_3$ was added during the 2000 study and 0.05 μM was added during the 2001 study. By adding two levels of $Na^{15}NO_3$, we are able to assess possible overestimation of the nitrate uptake rates. The initial and the incubated water samples were filtered onto precombusted (450°C, 4 h) glass fiber filters (Whatman GF/F). The filter papers were later treated with HC1 fumes for 2 h to remove carbonate and dried completely in a vacuum desiccator. Particulate organic carbon and nitrogen as well as the isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined by a mass spectrometer (ANCA-MASS 20-20 equipped with ANCA-SL preparation unit, Europa Scientific Ltd., Crewe, C.K.). Measurements of ^{13}C and ^{15}N atom% were reproducible at a precision of $\pm 0.0002\%$ and $\pm 0.0003\%$, respectively. Nitrate-based new production was converted to carbon by assuming Redfield ratio stoichiometry (i.e. $C = N \times 6.6 \times$ moles; Dugdale et al., 1989). Integrated primary production (IPP) and integrated new production (INP) were calculated by trapezoidal integration for the entire euphotic zone and are expressed as g C m^{-2}.

Three nutrient enrichment studies were conducted, including one at Station C in the 2000 cruise and one each at Stations 1 and C in the 2001 cruise. For each study, surface water was collected and was enriched with either N alone (+N), or P alone (+P), or both N and P (+NP); unamended sample served as the control (C). +N enrichment was carried out by adding 1 μM NO_3^- (KNO_3, Merk cat # 5063; with a maximum concentration of 0.0003% Fe), +P enrichment was 0.1 μM PO_4^{-3} (KH_2PO_4, Riedel-deHaen cat # 30407, with a maximum concentration of 0.0005% Fe) and the +NP enrichment was 1 μM NO_3^- plus 0.1 μM PO_4^{-3}. The amount of Fe introduced inadvertently because of chemical impurity was negligible, being <0.005 nM and <0.001 nM in the N and P enrichments, respectively. Acid-cleaned 20 l clear polycarbonate bottles (Nalgen, USA) were used as the incubation vessels. All bottles were placed in a large outdoor plastic container and incubated under the natural light for 6 days in the 2000 study and 5 days in the 2001 study. Incubation temperature was controlled by flowing through in situ surface seawater. Chlorophyll *a* concentration in each bottle was measured every 24 h to monitor

phytoplankton growth. During the 2000 studies at Station C, additional measurements of carbon uptake rate and nitrate uptake rate were carried out on the 3rd and 4th days following the same procedures used to measure production except an artificial light of 1.7×10^3 μE m^{-2} s^{-1} was employed. The light intensity was approximate to the average deck light level that was measured between 10:00 and 14:00 during the cruise. On the 5th day, water samples were collected and preserved by adding 3% neutralized formalin for later microscopic examination of phytoplankton species composition. Coccolithophores were examined by scanning electron microscopy (JSM6400, JEOL, Japan).

To evaluate the relative contribution of nitrogen from N_2-fixation, cell densities of two N_2-fixing planktonic cyanobacteria, *Trichodesmium* sp. and *Richelia intracellularis*, in the surface water of all six productivity stations were measured (Carpenter et al., 1999). Surface water of 2.3 l was filtered onto a 10 μm Nuclepore filter (47 mm in diameter). The density of *Trichodesmium* sp. was enumerated as units of trichomes per liter. *R. intracellularis* heterocysts and its symbiotic diatom cells were enumerated using a Zeiss epifluorescence microscope with green excitation (BP 510-560, FT 580, LP 590). N_2-fixation rates of the N_2-fixers were calculated based on their observed abundances and the fixation rates found in literatures. Two N_2-fixation rates for *Trichodesmium* were adopted: 14.9 pg N trichome^{-1} h^{-1} is the value obtained in a study conducted in the southern SCS (Saino, 1977), and 1.9 pg N trichome^{-1} h^{-1} was obtained in a study conducted in the central North Atlantic Ocean (McCarthy and Carpenter, 1979). The second value is a much conservative estimate. A fixation rate of 6.7 pg N cell^{-1} h^{-1} was used to calculate the N_2-fixation of *R. intracellularis* if its symbiotic hosts were *Hemiaulus* sp. (Villareal, 1991) or *Chaetoceros* sp. and a value of 2.1 pg N cell^{-1} h^{-1} was used when the hosts were *Rhizosolenia* sp. (Villareal, 1990). The summation of these N_2-fixations was used to represent the overall quantity of N_2-fixation in the study waters and was compared to the quantity of N from the nitrate uptake of phytoplankton.

3. Results

3.1. Spatial and temporal variation

The physiochemical properties measured in year 2000 and 2001 are generally similar. Surface water temperature measured in the March studies ranged from 23.46°C to 27.74°C (Tables 1 and 2). In average, water temperature was higher in 2001 than in 2000 but the difference is <2.5°C at any given station. There was a clear temperature gradient pattern in the SCS, increasing from northwest to southeast. Stratification index that measures the stability of the water column shows a reversed pattern, with high values in the southeast and low values in the northwest (Tables 1 and 2). The water column was in general more stratified in 2001 than in 2000 because of high surface temperature and low salinity observed in 2001. The euphotic zone depth was greater than the mixed layer depth (Tables 1 and 2) except for the northwestern stations. Surface irradiance was high; The PAR values during incubation were averaged 48.5 E m^{-2} d^{-1} in the 2000 study and 43.4 E m^{-2} d^{-1} in the 2001 study.

Surface nutrients were more abundant in 2000 than in 2001. Both nitrate and SRP were scarce in the upper euphotic layer. The range of surface $[NO_3 + NO_2]$ was 20–309 nM in 2000 and was 9–35 nM in 2001; The range of [SRP] was 24–88 nM in 2000 (Table 1) and 6–62 nM in 2001 (Table 2). Silicate was relatively abundant and its concentration ranged from 1.6 to 3.0 μM and 1.8 to 2.6 μM for 2000 and 2001, respectively. The ratios of $[NO_3 + NO_2]$/[SRP] (Tables 1 and 2) in the surface water were all far below the Redfield ratio N/P stoichiometry of 16:1, indicating the possibility of N-deficit. Surface $[NO_3 + NO_2]$ was negatively ($p<0.01$) correlated with the stratification index (Fig. 2). In contrast to the shallow water stations, the deep-water stations were more stratified and less productive. The surface chlorophyll *a* concentration ranged from 0.10 to -0.54 mg m^{-3} (Tables 1 and 2) and was positively ($p<0.01$) related to the surface $[NO_3 + NO_2]$ (Fig. 3). Phytoplankton was more abundant in 2000 than in 2001 (Fig. 3).

Table 1
The depth IPP (g C m^{-2} d^{-1}), INP (g C m^{-2} d^{-1}), *f*-ratio, chlorophyll *a*-specific primary production IPP/IChl (mg C mg Chl^{-1} d^{-1}), chlorophyll *a*-specific new production INP/IChl (mg C mg Chl^{-1} d^{-1}), chlorophyll *a* concentration of surface water $[Chla]_s$ (mg m^{-3}), $[NO_3 + NO_2]_s$ (nM), $[SRP]_s$ (nM), ratio of surface $[NO_3 + NO_2]$/[SRP], surface silicate concentration $[SiO_2]_s$ (μM), surface water temperature $Temp_s$ (°C), surface water salinity Sal_s (psu), depth of euphotic zone to 0.6% of surface light intensity Deu (m), depth of mixed layer Z_m (m), SI ($\times 10^2$ kg m^{-4}), bottom depth (m), surface light intensity during incubation PAR (E m^{-2} d^{-1}), light extinction coefficient K (m^{-1}) and *Trichodesmium* density ($\times 10^3$ filaments m^{-3}) measured at nine stations in the northern SCS during March 1–12, 2000

Variables	Station									Min–max
	1	2	3	4	A	B	C	D	E	
IPP	0.35	0.63	—	—	0.37	—	0.18	0.26	0.15	0.15–0.63
INP	0.07	0.36	—	—	0.20	—	0.07	0.12	0.09	0.07–0.36
f-ratio	0.20	0.57	—	—	0.54	—	0.39	0.46	0.60	0.20–0.60
IPP/IChl	11.60	25.77	—	—	14.65	—	10.08	15.13	9.20	9.20–25.77
INP/IChl	2.27	14.92	—	—	8.07	—	3.77	6.81	5.77	2.27–14.92
$[Chla]_s$	0.54	0.40	0.22	0.48	0.24	0.13	0.11	0.17	0.12	0.11–0.54
$[NO_3 + NO_2]_s$	152	135	70	309	22	84	74	20	29	20–309
$[SRP]_s$	83	79	51	88	45	33	24	60	43	24–88
$[NO_3 + NO_2]$/[SRP]	1.8	1.7	1.4	3.5	0.5	2.5	3.1	0.3	0.7	0.5–3.5
$[SiO_2]_s$	3.0	2.0	1.6	1.7	2.6	2.5	2.1	2.4	2.1	1.6–3.0
$Temp_s$	23.46	23.78	25.15	23.60	26.07	24.11	25.48	24.45	27.34	23.46–27.34
Sal_s	34.07	34.45	34.43	34.29	33.62	33.86	33.40	34.01	33.38	33.38–34.45
Deu	60	73	73	85	102	—	102	102	85	60–102
Zm	37	186	106	87	59	48	75	65	8	8–186
SI	0.47	0.10	1.37	0.91	2.49	1.45	2.02	1.86	2.69	0.10–2.69
Depth	69	651	758	2040	2495	2782	3791	3909	2897	69–3909
PAR	28.2	39.9	—	—	46.5	—	71.4	51.5	53.2	28.2–71.4
K	0.08	0.07	0.07	0.06	0.05	—	0.05	0.05	0.06	0.05–0.08
Trichodesmium	5.0	14.3	—	15.7	7.6	—	16.4	0	3.8	0–16.4

—: not available.

Springtime phytoplankton productivities in the SCS were low. Combined results of the two cruises showed that the magnitude of surface nitrate-based new production (NP, mg C m^{-3} d^{-1}) and primary production (PP, mg C m^{-3} d^{-1}) was positively related to surface $[NO_3 + NO_2]$ with regression equations as follows:

$$NP = -1.31 + 102.78\,[NO_3 + NO_2] \quad (R^2 = 0.81,\ p < 0.01),$$

$$PP = 6.67 + 138.64\,[NO_3 + NO_2] \quad (R^2 = 0.54,\ p < 0.01).$$

Water column integrated nitrate-based new productivity (INP) varied from 0.06 to 0.36 g C m^{-2} d^{-1}, whereas integrated primary productivity (IPP) ranged from 0.12 to 0.63 g C m^{-2} d^{-1} (Tables 1 and 2). INP and IPP measured in 2001 fell in the lower range of the 2000 measurements because water was more stratified and contained less nutrients in 2001 than in 2000. The *f*-ratio (INP/IPP) was in the range of 0.1–0.6. When the N_2-fixation rates of *Trichodesmium* and *R. intracellularis* were incorporated in the estimation of new production, the *f*-ratio changes only slightly because the contribution from N_2-fixation was very small.

Water at Station C was warm and stratified. N and P were scarce in the mixed layer (Fig. 4). Surface $[NO_3 + NO_2]$ was 74 nM and [SRP] was 24 nM in the 2000 study; and 29 and 49 nM, respectively in the 2001 study. The chlorophyll *a* concentration in the mixed layer was also low and showed a maximum at 60 m. The maximums of the nitrate-based new productivity and the primary productivity were found at the upper euphotic layer (Fig. 4), but not necessarily at the

Table 2
The depth IPP (g C m^{-2} d^{-1}), INP (g C m^{-2} d^{-1}), *f*-ratio, chlorophyll *a*-specific primary production IPP/IChl (mg C mg Chl^{-1} d^{-1}), chlorophyll *a*-specific new production INP/IChl (mg C mg Chl^{-1} d^{-1}), chlorophyll *a* concentration of surface water $[Chla]_s$ (mg m^{-3}), $[NO_3 + NO_2]_s$ (nM), $[SRP]_s$ (nM), ratio of surface $[NO_3 + NO_2]/[SRP]$, surface silicate concentration $[SiO_2]_s$ (μM), surface water temperature $Temp_s$ (°C), surface water salinity Sal_s (psu), depth of euphotic zone to 0.6% of surface light intensity Deu (m), depth of mixed layer Z_m (m), SI ($\times 10^2$ kg m^{-4}), bottom depth (m), light extinction coefficient *K* (m^{-1}) and *Trichodesmium* density ($\times 10^3$ filaments m^{-3}) measured at eight stations in the northern SCS during March 20–31, 2001

Variables	Station								Min–max
	1	2	3	A	B	C	D	E	
IPP	0.55	0.25	—	0.12	—	0.33	0.21	0.37	0.12–0.55
INP	0.08	0.06	—	—	—	0.06	0.06	0.15	0.06–0.15
f-ratio	0.14	0.24	—	—	—	0.19	0.26	0.41	0.14–0.41
IPP/IChl	49.23	13.19	—	6.71	—	13.02	8.81	15.56	6.71–49.23
INP/IChl	6.97	3.15	—	—	—	2.46	6.36	6.41	2.46–6.97
$[Chl]_s$	0.19	0.12	0.16	0.22	0.10	0.12	0.14	0.11	0.10–0.22
$[NO_3 + NO_2]_s$	18	35	24	19	9	29	23	10	9–35
$[SRP]_s$	62	62	55	50	57	49	38	6	6–62
$[NO_3 + NO_2]/[SRP]$	0.3	0.6	0.4	0.4	0.2	0.6	0.6	1.7	0.2–1.7
$[SiO_2]_s$	2.3	1.8	2.3	1.8	2.0	2.5	2.3	2.6	1.8–2.6
$Temp_s$	24.31	25.18	25.20	26.30	25.99	26.29	26.96	27.74	24.31–27.74
Sal_s	33.84	34.06	33.95	33.57	34.15	33.67	33.65	32.95	32.95–34.15
Deu	65	102	—	68	—	85	128	114	65–128
Zm	50	> 102	2	26	2	11	6	33	2–> 102
SI	2.23	1.37	1.83	2.47	1.97	2.40	2.66	3.07	1.37–3.07
Depth	69	714	779	2758	2840	3794	3911	3350	69–3911
K	0.06	0.05	—	0.075	—	0.06	0.04	0.045	0.04–0.075
Trichodesmium	13	6	44	6	0	8	13	22	0–44

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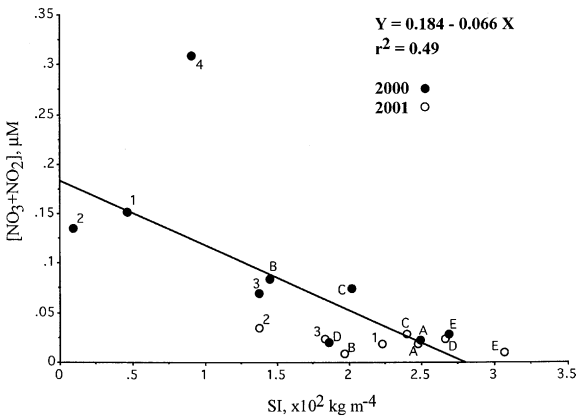


Fig. 2. Significant ($p < 0.01$) relationship between surface $[NO_3 + NO_2]$ and the SI of water measured on March 1–12, 2000 and on March 20–31, 2001 in the South China Sea. Numbers and alphabets are the station names.

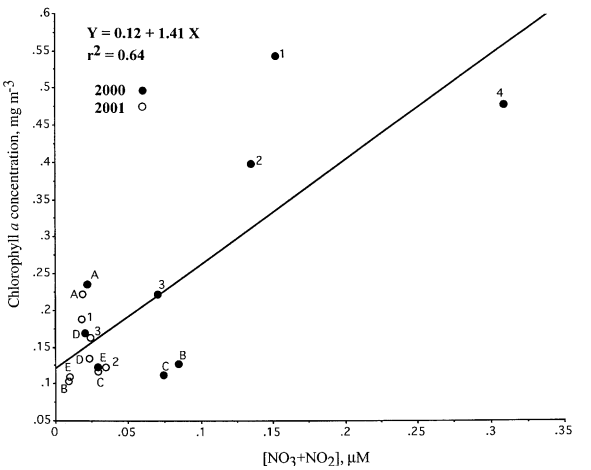


Fig. 3. Significant ($p < 0.01$) relationship between surface chlorophyll *a* concentration and surface $[NO_3 + NO_2]$ measured in March 2000 and in March 2001 in the South China Sea. Numbers and alphabets are the station names.

surface, indicating that both nutrient and illumination were critical for phytoplankton growth. Low nitrate-based new productivity (0.07 g C m^{-2} d^{-1} for 2000 and 0.06 g C m^{-2} d^{-1} for 2001) and primary productivity (0.18 and 0.33 g C m^{-2} d^{-1}, respectively) at Station C indicated

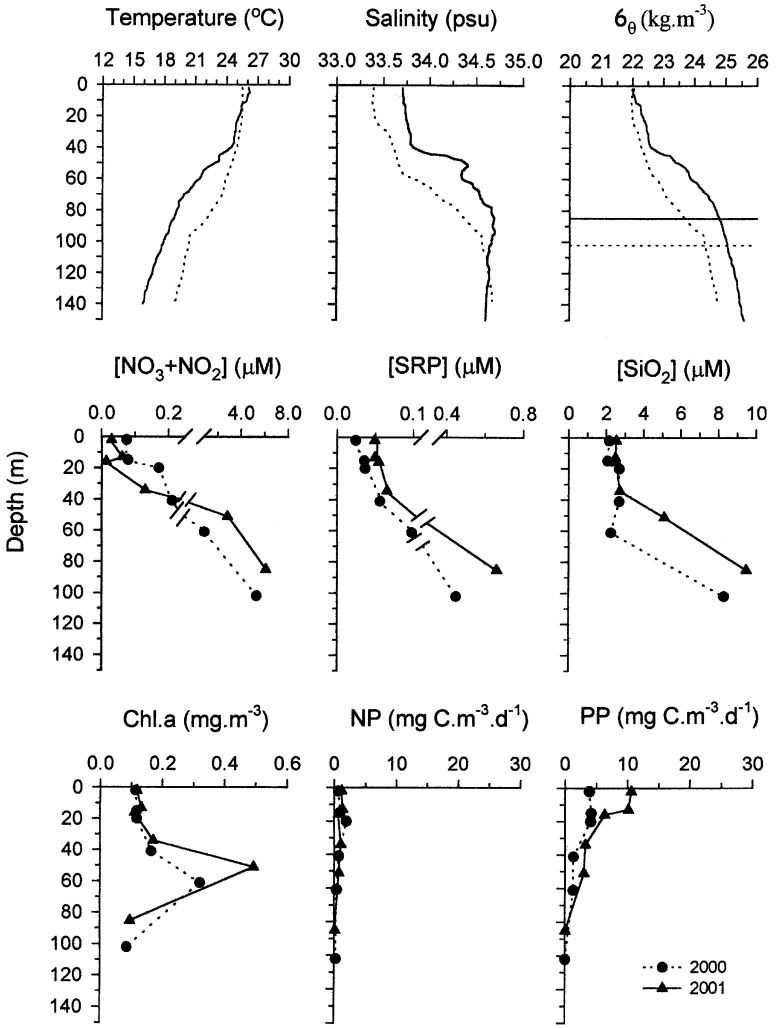


Fig. 4. Vertical profiles of water temperature, salinity, density (σ_θ), [$NO_3 + NO_2$], [SRP], silicate concentration [SiO_2], chlorophyll *a* concentration (Chl *a*), nitrate-based new production (NP) and primary production (PP) in Station C at the basin of the SCS, surveyed in March 2000 (in broken line) and March 2001, respectively.

that the basin station was relatively unproductive when compared to Station 1 that located on the shelf (Fig. 5).

3.2. Nutrient enrichment experiments

The results of the nutrient enrichment experiments clearly indicate that N is the nutrient limiting phytoplankton growth in the SCS. In the 2000 study at Station C, when enriched with +NP or +N, the chlorophyll *a* concentration, chlorophyll *a*-specific carbon uptake and chlorophyll *a*-specific nitrate uptake rates were all elevated (Table 3). In contrast, no enhancement in phytoplankton growth was observed in the control sample (no nutrient addition) and the +P enrichment. Chlorophyll *a* concentration, in response to +NP or +N enrichments, increased significantly at day 4, peaked at day 5 and declined subsequently (Fig. 6). The responses to the +P enrichment were not different from the unamended control. These results support the assertion that the surface seawater in the SCS basin where Station C was located was N-limited. Chlorophyll

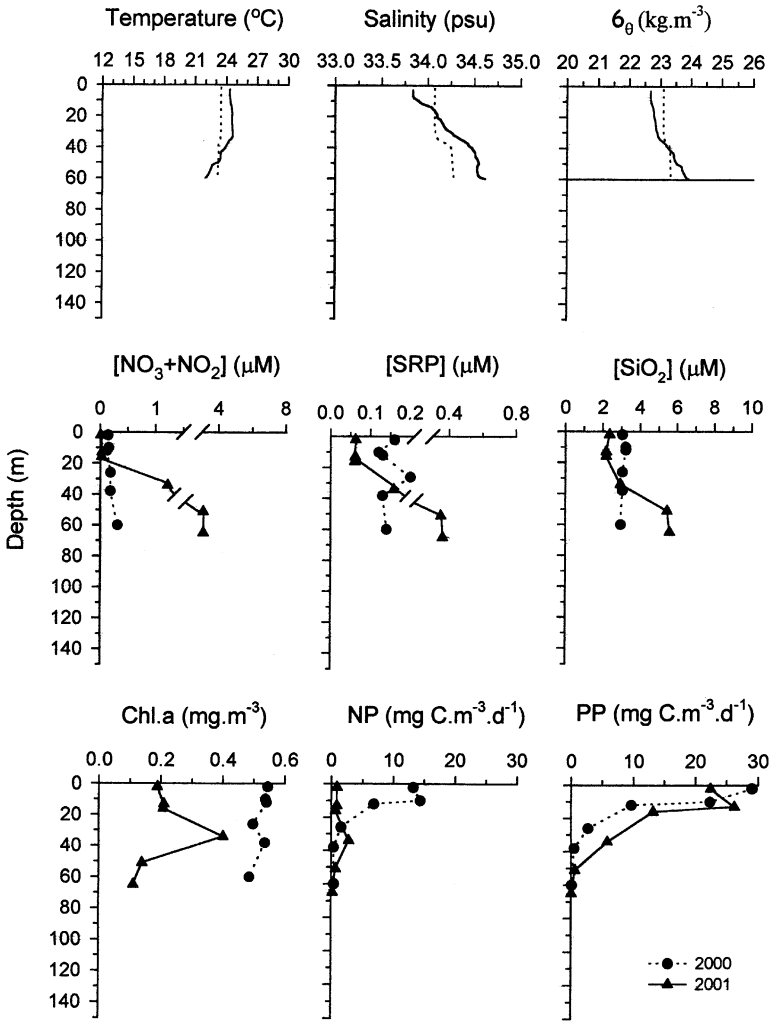


Fig. 5. Vertical profiles of water temperature, salinity, density (σ_θ), [$NO_3 + NO_2$], [SRP], silicate concentration [SiO_2], chlorophyll *a* concentration (Chl *a*), nitrate-based new production (NP) and primary production (PP) at Station 1 on the shelf of the South China Sea, surveyed in March 2000 (in broken line) and March 2001, respectively.

a-specific primary productions measured on day 4 for the +N (14.78 mg C mg Chl^{-1} h^{-1}) and for the +NP (11.40 mg C mg Chl^{-1} h^{-1}) were approximately twice in magnitude of those of the unamended control (6.93 mg C mg Chl^{-1} h^{-1}) and the +P enrichment (7.88 mg C mg Chl^{-1} h^{-1}). In terms of chlorophyll *a*-specific nitrate-based new production (Table 3), the differences between those with N enrichment (+N and +NP) and those without N enrichment (C and +P) are even greater, up to four times. Similar N-limitation was also observed during the 2001 study, at both Stations C and 1. In the 2001 studies, phytoplankton responded quickly to the +N or +NP enrichment. At both Stations 1 and C, the chlorophyll *a* concentration was significantly higher in the +N and +NP enrichments than the C and +P enrichments (Table 4). The results from these enrichment experiments strongly suggest that in the early spring nitrogen limitation was widespread in the SCS.

Table 3
Enhancement on days 3 and 4 of chlorophyll *a* concentration (Chl, mg m^{-3}), chlorophyll *a*-specific primary productivity (PP/Chl, mg C mg Chl^{-1} h^{-1}) and chlorophyll *a*-specific nitrate-based new productivity (NP/Chl, mg C mg Chl^{-1} h^{-1}) in responses to enrichments with no nutrient (C), 0.1 μM KH_2PO_4 (+P), 1 μM KNO_3 (+N) or 0.1 μM KH_2PO_4 + 1 μM KNO_3 (+NP) at Station C in the basin of the SCS in the enrichment experiments conducted in March 2000

Variables	Time (day)	Treatment			
		C	+P	+N	+NP
Chl	3	0.19	0.11	0.09	0.27
	4	0.14	0.13	0.56	0.88
PP/Chl	3	4.17	2.82	7.83	13.40
	4	6.93	7.88	14.78	11.40
NP/Chl	3	0.43	1.36	7.58	3.81
	4	2.29	2.71	6.09	9.43

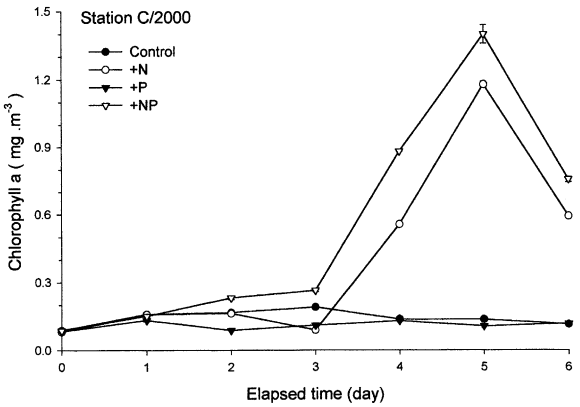


Fig. 6. Time-series change of chlorophyll *a* concentration in the nutrient enrichment experiment in which the surface water from Station C at basin of the SCS was enriched with either 0.1 μM of phosphate (+P), 1.0 μM of nitrate (+N), or both 1.0 μM nitrate and 0.1 μM phosphate (+NP) and incubated for 6 days during a cruise conducted in March 2000. No nutrient was added to the control group (C).

Changes in the phytoplankton community composition and cell density during the enrichment experiments also provide a qualitative evidence for N-limitation. The phytoplankton cell density increased significantly in the +N and +NP treatments, and on day 5 it reached a ratio of 6:3:1:1 for +NP:+N:+P:control (Table 5). On day 5, more centric diatoms were found in the +N and +NP treatments than in the C and +P treatments; and the reverse is true for the dinoflagellates (Table 5). Coccolithophorid population was more enhanced when N and P were added in combination than individually. *Emiliania huxleyi* was the most dominant coccolithophore species in the +NP enrichment, accounting for about 94% of total coccolithophorids. There was a small increase in the cell density of coccolithophores in response to the +N treatment. The cell size of these +N coccolithophorids was obviously smaller than that of the +NP cells (data not shown).

3.3. N_2-fixing cyanobacteria

The N_2-fixers were not abundant in the surface water of the SCS. There were 0–22.3×10^3 trichomes of *Trichodesmium* sp. and 0–21.5×10^3 of heterocystous *R. intracellularis* in a cubic meter of surface water in the 12 samples examined. The total N_2-fixation rate calculated from the abundance was at most, 0.34 μg N m^{-3} h^{-1}, assuming each trichome unit of *Trichodesmium* sp. fixing 14.9 pg N h^{-1}. The N_2-fixation rate fell to <0.20 μg N m^{-3} h^{-1} if the more conservative conversion rate of 1.9 pg N fixed h^{-1} was adopted. Comparing to the nitrate uptake rate of 6–301 μg N m^{-3} h^{-1}, the contribution of N_2-fixation was very little, $<3\%$ of the nitrate uptake rate at most (Table 6). The distribution of surface *Trichodesmium* was not statistically related to any of the environmental factors tested, including surface water temperature, salinity, [NO_3], [SRP], stratification index and mixed layer depth.

3.4. Possible errors of the nitrate uptake rate in the tracer-enrichment experiments

Our results show that the tracer enrichment did not enhance nitrate uptake. In the 2000 production experiments, two final concentrations of Na $^{15}NO_3$, 0.03 and 0.06 μM, were tested to assess possible over-estimation of nitrate uptake rates in the oligotrophic waters when the in situ nitrate concentration was below 0.3 μM. Fig. 7 shows the plot of NO_3 uptake rate when enriched with

Table 4
Enhancement of chlorophyll *a* concentration (Chl, mg m^{-3}, mean ±SE with $n = 2$), measured at Stations 1 and C in the 2001 study, in response to enrichments of no nutrient (C), 0.1 μM KH_2PO_4 (+P), 1 μM KNO_3 (+N) or 0.1 μM KH_2PO_4 + 1 μM KNO_3 (+NP)

Locality	Time (day)	Nutrient enrichment[a]			
		C	+P	+N	+NP
Station 1					
	1	$0.136^{a} \pm 0.005$	$0.113^{a} \pm 0.011$	$0.550^{b} \pm 0.069$	$0.510^{b} \pm 0.060$
	3	$0.092^{a} \pm 0.045$	$0.068^{a} \pm 0.001$	$0.320^{b} \pm 0.013$	$0.255^{b} \pm 0.027$
	4	$0.099^{ab} \pm 0.043$	$0.076^{a} \pm 0.004$	$0.202^{c} \pm 0.002$	$0.182^{bc} \pm 0.043$
	5	$0.084^{a} \pm 0.011$	$0.106^{a} \pm 0.015$	$0.267^{b} \pm 0.020$	$0.287^{b} \pm 0.078$
Station C					
	1	$0.119^{a} \pm 0.021$	$0.089^{a} \pm 0.006$	$0.188^{b} \pm 0.009$	$0.199^{b} \pm 0.033$
	3	$0.155^{b} \pm 0.005$	$0.077^{a} \pm 0.004$	$0.383^{c} \pm 0.032$	$0.334^{c} \pm 0.021$
	4	$0.127^{a} \pm 0.035$	$0.098^{a} \pm 0.022$	$0.494^{b} \pm 0.045$	$0.725^{c} \pm 0.127$
	5	$0.110^{a} \pm 0.033$	$0.065^{a} \pm 0.004$	$0.591^{b} \pm 0.068$	$0.318^{a} \pm 0.160$

Stations 1 and C were located on the shelf and the basin of the SCS, respectively. The initial chlorophyll *a* concentration before nutrient supplementation was 0.157 ± 0.006 mg m^{-3} at Station 1 and 0.111 ± 0.015 mg m^{-3} at Station C.

[a] Means in the row with different superscript letter are significantly different ($p < 0.05$) when compared by the Duncan's new multiple range test.

Table 5
Major group composition (%) and cell density ($\times 10^4$ cells l^{-1}) of phytoplankton community on the 5th day of the nutrient enrichment experiment conducted in March 2000

Phytoplankton	Treatment			
	C	+P	+N	+NP
Diatoms				
Centric	2.5	4.8	9.7	8.2
Pennate	28.4	24.0	32.5	27.2
Dinoflagellates	17.6	23.1	12.1	9.3
Coccolithophorids	35.2	17.7	28.0	43.8
Others	16.3	30.4	17.7	11.5
Cell density	0.74	0.86	2.51	5.53

No enrichment (C) as well as 0.1 μM KH_2PO_4 (+P), 1 μM KNO_3 (+N) and 0.1 μM KH_2PO_4 + 1 μM KNO_3 (+NP) were added to surface water of Station C at the basin of the SCS.

0.03 μM Na $^{15}NO_3$ against those enriched with 0.06 μM of Na $^{15}NO_3$. All data points scatter closely along the 1:1 line (Fig. 7), indicating that nitrate uptake rate was not affected by the amount of NO_3 added, at least when the enrichment level was <0.06 μM. Further correction of the nitrate uptake rate to zero nitrate enrichment was deemed necessary. Nitrate-based new production measured in the 2000 study, consequently, was calculated based on the data set from the 0.03 μM enrichment.

It is also unlikely that nitrate uptake rate was underestimated due to nutrient exhaustion. The proportion of ^{15}N uptake during the 3-h incubation was mostly less than 15% (in 29 out of 34 cases) or overly less than 35%. The nutrient exhaustion did not occur during the incubations.

4. Discussion

Phytoplankton growth in the SCS during early spring is limited by nitrogen. This conclusion is drawn from several lines of evidence, including: (1) the low in situ $[NO_3 + NO_2]$; (2) the low $[NO_3 + NO_2]$/[SRP] when compared to the Redfield N/P Ratio of 16:1; (3) enhanced phytoplankton growth in response to N enrichment but not to P enrichment; and (4) the significantly positive relationship between surface $[NO_3 + NO_2]$ and the magnitudes of nitrate-based new production and primary production. The known nitrogen limitation region in the western equatorial Pacific covering a wide area east of 130°E (Barber and Chavez, 1991) could, thus, have been extended westward based on our results. Unlike the northern Atlantic Ocean, at the similar latitudes of the SCS, where N is excessive relative to P and the contribution of N_2-fixation to new N is significant

Table 6
Abundance of *Trichodesmium* sp. ($\times 10^3$ trichomes m^{-3}) and *R. intracellularis* in the surface water of the SCS and their estimated nitrogen fixation rate (μ g N m^{-3} h^{-1}) as well as the nitrate uptake rate (μg N m^{-3} h^{-1}) from the in situ measurement

Year/St	*Trichodesmium*	*R. intracellularis*			N_2-fixation rate[a]		NO_3 uptake	N_2-fixing/NO_3 uptake (%)
		Chaetoceros	*Hemiaulus*	*Rhizosolenia*	Low value	High value		
2000/1	5.0	0	0	0	0.01	0.07	301	<0.02
2	14.3	0	4.2	0	0.06	0.24	263	0.01–0.09
A	7.6	0.4	10.5	8.8	0.11	0.20	31	0.35–0.65
C	16.4	6.7	3.4	0	0.10	0.31	13	0.77–2.38
D	0	0	0	0	0	0	39	0
E	3.8	5.0	0.8	2.5	0.05	0.10	6	0.83–1.67
2001/1	13.2	0	21.5	0	0.17	0.34	14	1.21–2.43
2	5.8	0	0	0	0.01	0.09	21	0.05–0.43
A	5.8	0	0	0	0.01	0.09	52	0.02–0.17
C	8.3	0	0	0	0.02	0.12	27	0.07–0.44
D	13.2	0	0	4.1	0.03	0.21	32	0.09–0.66
E	22.3	0	0	0	0.04	0.33	47	0.09–0.70

[a] Based on a fixing rate of 14.9 pg N $trichome^{-1}$ h^{-1} for *Trichodesmium* sp. (Saino, 1977) for high value and 1.9 pg N $trichome^{-1}$ h^{-1} (McCarthy and Carpenter, 1979) for low value; and 6.7 pg N $cell^{-1}$ h^{-1} was applied to *R. intracellularis* when they were symbiotic in *Hemiaulus* sp. (Villareal, 1991) and *Chaetoceros* sp. or 2.1 pg N $cell^{-1}$ h^{-1} in *Rhizosolenia* sp. (Villareal, 1990).

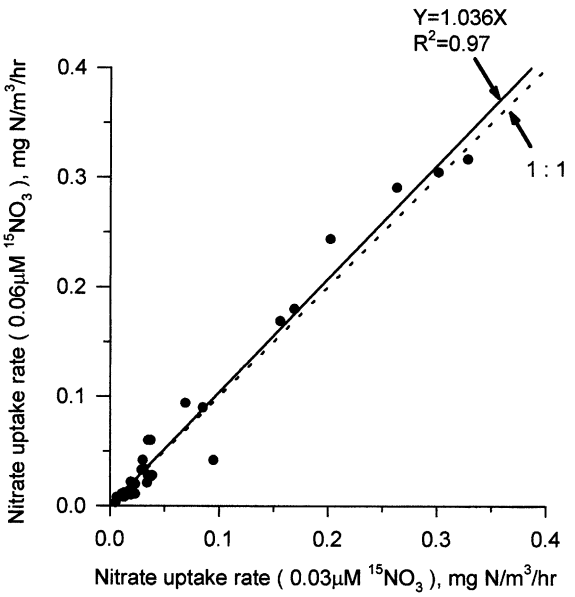


Fig. 7. Regression analysis of nitrate uptake rates between responses to 0.03 μM enrichment and to 0.06 μM of $Na^{15}NO_3$ enrichment. The 1:1 line indicates equal nitrate uptake.

(Michaels et al., 1996; Wu et al., 2000), the SCS is nitrogen deficient and N_2-fixation is very limited.

At the subtropical Station Aloha (22°45′N, 158°W) in the eastern North Pacific, Karl et al. (1997) observed that the ecosystem shifts from N- to P-limited when N_2-fixation of *Trichodesmium* sp. becomes a significant N source. Our study shows that production in the western North Pacific is limited by N not by P. During the study of two consecutive early springs, N_2-fixation of the planktonic cyanobacteria was not found to be a significant N source.

Nitrogen-fixing cyanobacteria need iron as a co-factor for nitrogenase that modulates the rate of N_2-fixation (Wu et al., 2000). Vast input of iron from atmospheric dust stimulates the nitrogen fixation in the tropical Atlantic Ocean (Prospero et al., 1996). In the eastern Equatorial region where other nutrients are sufficient, an eolian iron flux of <1 mg m^{-2} yr^{-1} was found to limit the biological productivity. The atmospheric flux of iron in the northern SCS is >1000 mg m^{-2} yr^{-1} (Duce and Tindale, 1991) because of the airborne dust from the Asian continent. The substantial influx of iron may infer that the N-deficit in the SCS is not caused by the iron shortage. On the other hand, however, the solubility of iron particles is decided by the sufficiency of organic ligands in the water (Wu et al., 2001). Phytoplankton uses only the soluble forms of iron. The shortage of the organic ligands makes iron

unavailable to phytoplankton even when airborne supply of iron is high. The abundance of the organic ligands and their effects on iron availability in the SCS are not known.

Inhibition of the N_2-fixation process by the iron shortage in open oceans has been suggested to reconcile the discrepancy between N_2-fixation and denitrification (Falkowski, 1997) and result in a widespread nitrate deficit. The results of a recent model study (Tyrrell, 1999), however, did not support the iron-limitation hypothesis. It, in turn, predicted a persistent N-deficit even when iron is in surplus. Tyrrell (1999) suggested that further field study in iron-rich vs. iron-poor systems is needed to verify whether nitrate deficit would persist when the ambient iron concentrations are high. The profiles of our study apparently meet the criteria and fit to test this argument. Our results indicate that N-deficit occurs in the SCS where iron is supposedly abundant, assuming that the high eolian iron flux would result in high dissolved iron concentration.

Both *Trichodesmium* and *Richelia* were scarcely distributed in the oligotrophic SCS during the study periods. *Trichodesmium* sp. (Carpenter and Price, 1977) and *Richelia*/*Hemiaulus* (Carpenter et al., 1999) often bloom in the tropical Atlantic Ocean. *Richelia*/*Hemiaulus* bloom in the Amazon River plume, and the blooms are modulated by the relative abundance of silicate and iron in the river water. The silicate concentration was relatively high in the SCS (Tables 1 and 2). The nutrient enrichment studies demonstrate clearly that diatoms would thrive in response to nitrate enrichment without the need of silicate supplement (Table 5). A recent study in the central Atlantic Ocean (Sañudo-Wilhelmy et al., 2001) shows that P and light might control N_2-fixation under the iron-rich conditions. In the SCS, P and light conditions are unlikely to hamper the growth of the N_2-fixing cyanobacteria. Surface seawater of the SCS is usually high in $[SRP]/[NO_3 + NO_2]$ (Tables 1 and 2) and does not respond to P enrichment (Tables 3 and 4), indicating the ample availability of P in this light-intensive tropical ocean.

Trichodesmium thrives in regions where water is generally above 20°C (Marumo and Nagasawa, 1976), stratified or lowly turbulent (Carpenter and Price, 1976), P-sufficient (Doremus, 1982; Carpenter and Price, 1977; Sañudo-Wilhelmy et al., 2001) and iron-sufficient (Rueter, 1983). All environmental factors during our studies appeared favorable for the growth of *Trichodesmium* except for the calm weather. *Trichodesmium* usually bloom when the weather is sunny and calm (Bell et al., 1999). In the study waters, where the northeastern monsoon usually prevails in March, we recorded a steady northeasterly with an average wind speed of 11.8 m s^{-1} during the year 2000 cruise. The windy weather and the ensuing turbulent water column did not favor the growth of *Trichodesmium*. Relatively speaking, although the mixed layer in springtime is prone to be deep, less stratified, and high in surface nitrate concentration, the surface water of the SCS as we investigated in the two consecutive early springs was N deficient. No prevailing wind was recorded during the survey of March 2001. The wind speed ranged 3–15 m s^{-1}. The relatively calm weather and stratified water column (Tables 1 and 2), however, did not result in a dense *Trichodesmium* population. Weather and water column stratification, thus, did not appear as controlling factors in our study since the density of *Trichodesmium* responded to neither of them. *Trichodesmium* is a slow grower (Orcutt et al., 2001) and the time needed for the accumulation of its biomass is consequently considerable. It is possible that population of *Trichodesmium* might flourish in good time or most likely in summer, as seen in many subtropical marine ecosystems where calm weather and highly stratified water column persist. A comprehensive time-series study in the SCS that investigates the interaction of nutrient and plankton dynamics is probably required to better understand the controlling factors of new production and the role of iron in these biogeochemical trajectories in this tropical marginal sea.

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