Dissolved inorganic phosphorus, dissolved iron, and *Trichodesmium* in the oligotrophic South China Sea

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[1] Dissolved inorganic phosphorus (DIP) concentrations in the oligotrophic surface waters of the South China Sea decrease from ~20 nM in March 2000 to ~5 nM in July 2000, in response to seasonal water column stratification. These minimum DIP concentrations are one order of magnitude higher than those in the P-limited, iron-replete stratified surface waters of the western North Atlantic, suggesting that the ecosystem in the South China Sea may be limited by bioavailable nitrogen or some trace nutrient rather than DIP. Nutrient enrichment experiments using either nitrate, phosphate or both indicate that nitrogen limits the net growth of phytoplankton in the South China Sea, at least during March and July 2000. The fixed nitrogen limitation may result from the excess phosphate (N:P<16) transported into the South China Sea from the North Pacific relative to microbial population needs, or from iron control of nitrogen fixation. The iron-limited nitrogen fixation hypothesis is supported by the observation of low population densities of *Trichodesmium spp.* ($<48 \times 10^3$ trichomes/m^3), the putative N_2 fixing cyanobacterium, and with low concentrations of dissolved iron (~0.2–0.3 nM) in the South China Sea surface water. Our results suggest that nitrogen fixation can be limited by available iron even in regions with a high rate of atmospheric dust deposition such as in the South China Sea. *INDEX TERMS:* 1065 Geochemistry: Trace elements (3670); 4845 Oceanography: Biological and Chemical: Nutrients and nutrient cycling; 1615 Global Change: Biogeochemical processes (4805); *KEYWORDS:* iron distribution, phosphate and nitrogen, nutrient cycles, South China Sea, nitrogen fixation

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1. Introduction

[2] Nitrogen bioavailability is believed to limit primary production in the present-day oligotrophic oceans [*Ryther and Dunstan*, 1972; *Codispoti*, 1989]. Dinitrogen (N_2) fixation, a conversion of the otherwise unavailable pool of N_2 to ammonia by diazotrophic organisms such as *Trichodesmium sp*, represents an important source of new nitrogen to the ocean's euphotic zone [*Karl et al.*, 1997]. Contemporary N_2 fixation is thought to be limited by the availability of iron (Fe), an important cofactor for the enzyme nitrogenase [*Raven*, 1988; *Falkowski*, 1997; *Wu et al.*, 2000]. However, direct evidence for Fe limitation of N_2 fixation in the ocean is lacking. By contrast, N_2 fixation in the Fe-replete subtropical North Atlantic is thought to be limited by available phosphorus (P) [*Sanudo-Wilhelmy et al.*, 2001].

[3] Iron versus phosphorus limitation of N_2 fixation can be diagnosed from dissolved inorganic phosphate (DIP) distributions in stratified oligotrophic surface waters. Surface water DIP is consumed by microorganisms, and supplied by DIP input from subsurface water and by in situ microbial degradation of phosphorus-containing organic matter in the surface water (some microorganisms can assimilate certain dissolved organic phosphorus (DOP) compounds directly or indirectly via cell surface-associated enzymes (K. Bjorkman and D.M. Karl, Bioavailability of dissolved organic phosphorus in the euphotic zone of Station ALOHA, North Pacific Subtropical Gyre, submitted to *Limnology and Oceanography*, 2002). If phytoplankton assimilate nitrogen and phosphorus at a fixed ratio (16N:1P), excess nitrogen input from N_2 fixation increases biological DIP uptake which depletes surface water DIP and alters the dissolved N:P ratio.

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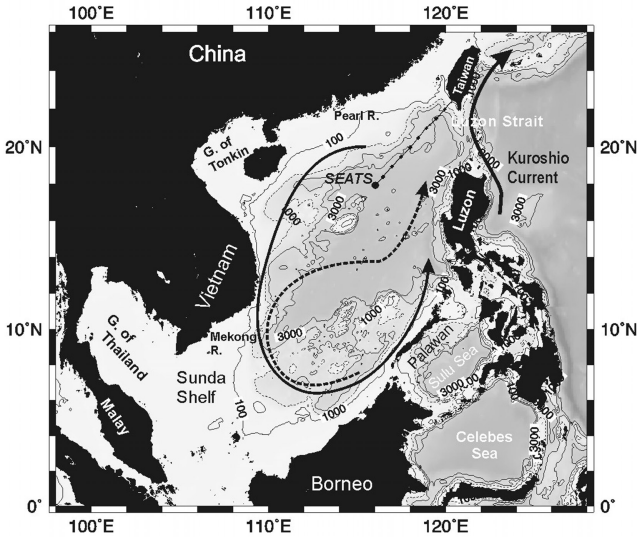


Figure 1. Map with isobaths (in meters) of the South China Sea showing sampling station. The main site of SEATS (with Station S1 at 18°N, 116°E) is indicated by the solid circle. The solid and the dashed curves with arrows inside the SCS indicate the winter and summer jets, respectively. Also shown is the main path of the Kuroshio, which intrudes into the SCS from time to time, especially in winter.

[4] During the past decade in the subtropical Northeast Pacific Ocean, climatically driven increases in surface ocean stratification and resulting increases in N_2 fixation have decreased surface water soluble reactive phosphate (SRP) concentrations by 50–75% [*Karl and Tien*, 1992; *Karl et al.*, 1997] (As an operationally defined parameter, SRP includes DIP and labile organic phosphate that is reactive toward the colorimetric Mo-Blue analytical procedure for P, although organic P species are often only a small portion (10%) of total SRP). Despite this decrease, current levels of SRP in the surface waters of the subtropical North Pacific are one order of magnitude higher than those of DIP in the western subtropical North Atlantic [*Wu et al.*, 2000], so further decreases may be anticipated. The difference in surface water DIP concentrations between the two oceans is hypothesized to result from Fe limitation of N_2 fixation in the North Pacific [*Wu et al.*, 2000].

[5] The South China Sea (SCS) (Figure 1) is located in the tropical western North Pacific. Its central gyre is warm, permanently stratified and oligotrophic. Dust input from the Gobi Desert supplies substantial particulate Fe to the SCS surface water [*Duce et al.*, 1991], only a fraction of which may be bioavailable. Because these conditions in the SCS (warm, permanently stratified, oligotrophic, and dust rich) all favor N_2 fixation [*Capone et al.*, 1997; *Karl et al.*, 2002], available P in the SCS surface water should be biologically depleted to a limiting level (<1 nM). However, we observed relatively high concentrations of DIP, low ratios of dissolved nitrate plus nitrite (DNN) to DIP, low concentrations of dissolved Fe, and low abundances of the N_2 fixing cyanobacterium, *Trichodesmium*, in the surface waters of the SCS. Although the deposition rate of total Fe (mostly from eolian input) is substantial [*Duce et al.*, 1991], our data suggest that N_2 fixation in the SCS may still be limited by available Fe.

2. Study Area

[6] The SCS is an enclosed basin in the tropical-subtropical western North Pacific (Figure 1). Its subsurface waters exchange with Western Philippine Sea (WPS) through Luzon Strait with a sill depth of ~1900 m. As the WPS water enters the SCS from the Luzon Strait at depths between 1500–1900 m, the deeper portion of this water sinks to the bottom of the basin (as the deep water source in the basin), and the upper portion mixes (across isopycnal surfaces) upward and eventually flows out of the basin at intermediate depths (~500–1000) [*Gong et al.*, 1992]. Radiocarbon (^{14}C) data and vertical mixing models suggest that the residence time of WPS water in the basin is on the order of 40 to 115 yrs [*Wang*, 1986; *Broecker et al.*, 1986].

[7] The SCS upper water column is permanently stratified with an annual mean mixed layer depth of ~40 m. The surface water circulation is strongly influenced by monsoonal forcing, with cyclonic flow during the northeast monsoon in winter and partially reversed flow pattern during the southwest monsoon in summer. The Kuroshio, which flows northward to the east of the Luzon Island, intrudes into the SCS through the Luzon Strait from time to time, especially in winter [*Shaw*, 1991]. The northeast monsoon carries a substantial amount of Fe-rich eolian dust from Gobi Desert to the SCS [*Duce et al.*, 1991]. Diapycnal mixing is enhanced by the strong internal waves in the SCS basin. The mixing eliminates the oxygen minimum layer at depth and increases macronutrient concentrations in the upper 600 m of the SCS [*Gong et al.*, 1992].

3. Sample Collection and Analysis

[8] Seawater samples for nutrient analyses were collected at the Taiwan South East Asia Time series (SEATS) station S1 (18 °N, 116 °E) on three cruises in March (SEATS 9), May (SEATS 10) and July (SEATS 11) 2000 using 5-L Niskin water bottles attached to a standard hydrowire. The seawater samples were immediately frozen (without filtration) in polyethylene bottles using liquid N_2 and were stored at −10°C until analyzed. DIP was measured by a high sensitivity MAGIC method (for <20 nM) and by standard colorimetric technique (for >20 nM) using a 5 cm light-path spectrophotometer cell [*Wu et al.*, 2000]. Quantifications were made by comparing sample absorbance with standard curves determined by phosphate additions to a P-depleted surface seawater sample which was collected from the SCS and stored in polyethylene bottles at room temperature for more than 1 month before use. The detection limit for the MAGIC method is ~0.4 nM for a 50-fold preconcentration procedure (using 150 μl of 1M NaOH per 50 ml seawater sample), and ~20 nM without preconcentration [*Wu et al.*, 2000]. Because the samples for DIP measurements are not filtered, any reactive P that is released from microbial cells during the freezing process may also contribute to the measured DIP value.

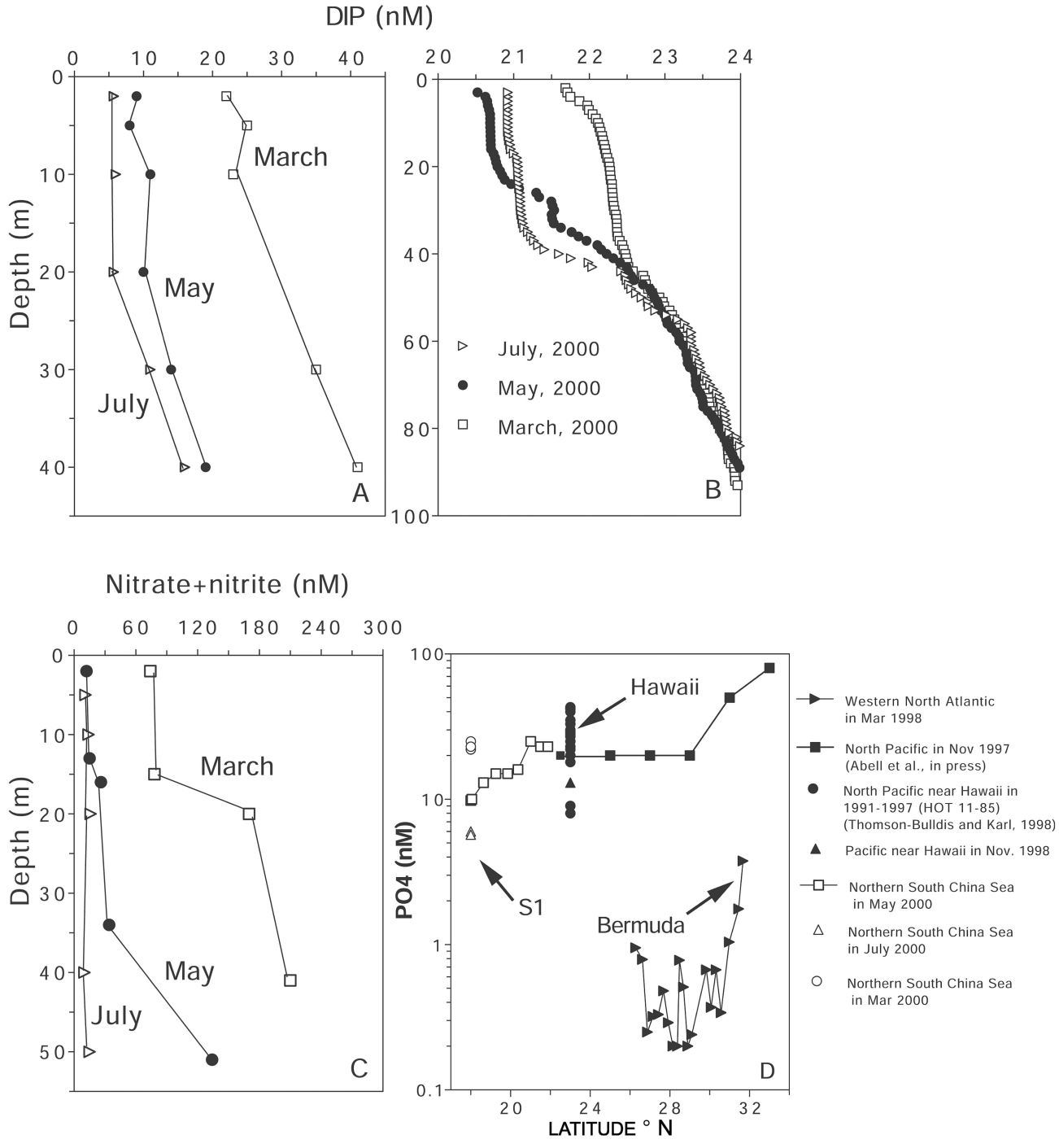


Figure 2. Vertical profiles of (a) DIP, (b) water density, and (c) DNN in the SCS (station S1, 18°N, 116°E) and (d) the comparison of surface water DIP between SCS, North Pacific, and North Atlantic Oceans.

[9] Dissolved nitrate plus nitrite (DNN) concentrations of the upper water column were determined by the high-sensitivity chemiluminescent method (detection limit 1 nM [*Garside*, 1982]); whereas those of the deep water were measured by standard colometric methods with a detection limit of ~30 nM. Total organic phosphorus (TOP) and nitrogen (TON) were measured with standard colorimetric methods for UV irradiated samples (1000 W, 2 h for TOP and 24 h for TON) [*Armstrong and Tibbets*, 1968]. We define the difference between TOP and DIP as dissolved organic phosphate (DOP) and the difference between TON and DNN as dissolved organic nitrogen (DON). Based on this definition, both DOP and DON would also contain contributions from particulate P and N in unfiltered samples.

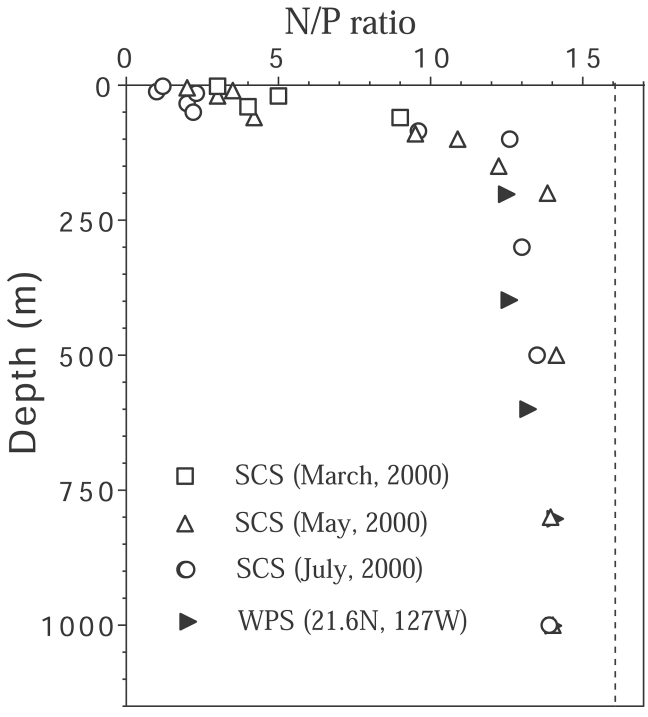


Figure 3. Vertical profiles of DNN:DIP ratio in the SCS and the comparison between SCS and WPS.

[10] Samples for Fe concentration measurement were collected with the MITESS sampler [*Bell et al.*, 2002; *Dickey et al.*, 1998] suspended on a standard hydrowire. The samples were filtered through 0.4 μm Nuclepore polycarbonate filters inside a class-100 clean bench on board the ship. Both the filtrate and unfiltered replicates were acidified (to pH 2.2) with 1 ml Vycor HCl (6N, Fe blank = 4 pmole Fe per 1 ml HCl addition), stored at room temperature, and analyzed for Fe by isotope dilution-ICPMS [*Wu and Boyle*, 1998]. This ICPMS method uses 1.3 ml sample and has a detection limit of ~0.03 nM. Fe measured by this method would include inorganic and organic Fe species that are present as Fe^{3+} at pH 2.2, as well as Fe inside small particles and organic materials that can be co-precipitated by the $Mg(OH)_2$ method.

[11] To assess the nutrient status of phytoplankton growth, enrichment experiments were conducted on board during March and July, 2000. Nutrient-poor surface water was enriched with either 1 μM nitrate (KNO_3, Merck 5063; with a maximum concentration of 0.0003% Fe by weight), 0.1 μM phosphate (KH_2PO_4, Riedel-deHaen 30407; with a maximum concentration of 0.0005% Fe by weight) or a combination of nitrate and phosphate (1 μM NO_3^- + 0.1 μM PO_4^{3-}). Un-amended samples served as controls. The amount of Fe potentially introduced by these nutrient enrichments was negligible, being <0.005 nM in the +N enrichment and <0.001 nM in the +P enrichment. All enrichment experiments were conducted under natural light for 4–5 days in 20-l acid-cleaned clear polycarbonate bottles. Temperature was maintained by circulating surface seawater through an incubator holding the containers. Phytoplankton growth was monitored by measuring the chlorophyll *a* concentration on acetone-extracted samples [*Strickland and Parsons*, 1972] with a Hitachi model F3010 fluorescence spectrophotometer (Hitachi Co., Tokyo, Japan).

[12] To evaluate the growth status of *Trichodesmium* and its potential contribution to the nitrogen budget, cell densities of *Trichodesmium spp*. were measured [*Carpenter et al.*, 1999]; samples were collected in March, May and July 2000 and in March 2001. A volume of seawater (2.3 l) was filtered through a 10 μm Nuclepore filter (47 mm diameter), and *Trichodesmium spp.* colonies were enumerated using a Zeiss epifluorescence microscope under green excitation (BP 510-560, FT 580, LP 590). N_2 fixation rates were estimated using literature values of mean N_2 fixation per trichome. Two N_2 fixation rates for *Trichodesmium* were adopted: 14.9 pg N trichome^{-1} hr^{-1} measured in the southern South China Sea [*Saino*, 1977] and 1.9 pg N trichome^{-1} hr^{-1} measured in the central North Atlantic Ocean [*McCarthy and Carpenter*, 1979]. The latter value served as a conservative estimate, especially considering the fact that *Trichodesmium* is only one of several potentially important N_2 fixers. These estimates were then compared to

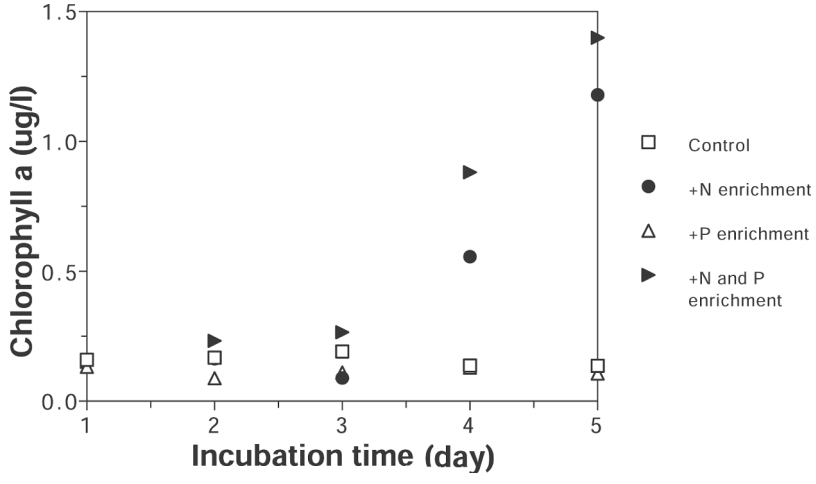


Figure 4. Changes of Chl *a* versus incubation time in the nutrient enrichment experiments.

the measured rates of nitrate uptake using ^{15}N-labeled tracer [*Dugdale and Wilkerson*, 1986; *Chen et al.*, 1999].

4. Results and Discussion

4.1. DIP5 Distribution

[13] DIP concentrations in SCS surface water (~2 m depth) show a systematic variation (Figure 2a) in response to surface water stratification. As the surface mixed layer depth at station S1 decreases from ~40 m in March to ~20 m in May and July (Figure 2b), DIP concentrations decrease from ~20 nM to ~5 nM (Figure 2a), and DNN concentrations decrease from ~70 nM to ~10 nM (Figure 2c). The molar ratios of DNN:DIP in the euphotic zone (2:1 to 4:1, Figure 3) are much lower than the Redfield N:P stoichiometry of 16:1, implying a N deficiency for phytoplankton growth.

[14] The lowest DIP concentration that we observed in SCS surface water (~5 nM, Figure 2a) is similar to those observed in the oligotrophic North Pacific central gyre, but is one order of magnitude higher than the approximate 0.4 nM DIP concentrations measured for the stratified surface waters of the western North Atlantic (Figure 2d). If we assume that the DIP concentrations in the western North Atlantic represent a lower threshold limit of DIP for picoplankton growth, the relatively higher concentrations of DIP in the SCS would imply that P is not a limiting nutrient in the SCS ecosystem. As a preferred form of P for microbial growth, DIP concentrations in stratified surface waters should be biologically depleted to limiting levels, if N is readily available and if all other required major and trace nutrients are present in excess relative to cell needs. The relatively high concentrations of DIP that we observed in the surface waters of the SCS suggest that P should not limit phytoplankton growth in the SCS. This result is consistent with our nutrient enrichment experiments (Figure 4). The addition of inorganic N alone or N plus P produced a two- to three-fold increase in chlorophyll a concentration compared to the unammended controls; the +P enrichment treatment likewise showed no chlorophyll increase (Figure 4). If P is the limiting factor, the addition of P would enhance algal biomass. The lack of response by the P addition treatment, and the increase of biomass induced by the N addition support the hypothesis that N is a limiting factor for phytoplankton growth in the SCS.

[15] The nitrogen limitation appears to result from the selective surface water enrichment by excess P introduced to the SCS surface water from upward nutrient supply and from in situ nutrient recycling. DNN to DIP ratios in the upper 1000 m of the SCS range from approximately 1 to 10 (Figure 3) which are lower than the Redfield molar ratio of 16N:1P in plankton. Even below the euphotic zone to the depths of 1000 m, the DNN:DIP ratios do not exceed 14:1 (Figure 3). Because of these low ratios, as the subsurface water is mixed to the surface, microbial growth at a N:P ratio of 16:1 will deplete N before P, a situation consistent with the observed near-surface excess of DIP concentrations in the euphotic zone. The low DNN:DIP ratios in the SCS subsurface waters appear to originate from the WPS by water exchange through Luzon Strait. As the sole source of deep water to the SCS, the WPS subsurface waters contain a low ratio of DNN:DIP because of a surplus of water column denitrification over N_2 fixation in the North Pacific [*Wu et al.*, 2000; *Deutsch et al.*, 2001].

[16] In addition to upward P supply, excess phosphorus may be introduced in situ by nutrient cycling in the stratified surface mixed layer. If organic nitrogen compounds that are released by algae are more resistant to microbial degradation than organic phosphorus compounds [*Jackson and Williams*, 1985; *Abell et al.*, 2000], refractory dissolved organic nitrogen (DON) would be preferentially accumulated relative to dissolved organic phosphorus (DOP) in the surface water, leading to a higher than Redfield stoichiometric DON:DOP ratio of ~28:1 (Figure 5). As this high N:P ratio dissolved organic matter is exported down to the subsurface, an excess DIP equivalent to ~70% of downward DOP export would be left in the euphotic zone, if organic matter produced by phytoplankton has a N:P ratio of 16:1, and if the dissolved organic matter (DOM) has a N:P ratio of 28:1.

4.2. *Trichodesmium* Abundance

[17] The N limitation occurs concurrently with low abundance of the large colonial N_2 fixing cyanobacterium *Trichodesmium* [*Capone et al.*, 1997] in the SCS surface water. At station S1, the maximum concentrations of *Trichodesmium* (48 trichomes/L of seawater, Table 1) is one order of magnitude lower than that in the East China Sea (600 trichomes/L [*Chang et al.*, 2000]) and is three orders of magnitude lower than the maximum reported in the Caribbean Sea [*Carpenter and Price*, 1976]. Most of the *Trichodesmium* in the SCS surface water is present as individual trichomes; colonial forms were rare and when present were composed of very few trichomes, usually less than 10 trichomes per colony.

[18] Although free trichomes of *Trichodesmium* can also fix N_2, they do so with a lower per cell rate than when present in the colony morphology [*Letelier and Karl*, 1998]. The upper bound of N_2 fixation derived from the observed *Trichodesmium* abundance is 0.72 μg N m^{-3} hr^{-1}, assuming that each unit trichome of *Trichodesmium spp.* fixes 1.9 pg N hr^{-1}. At this net rate (~30 nM N/month), it would take ~5 years for N_2 fixation to deplete surface water excess DIP (~138 nM as estimated from mass balance discussed below) to the levels that we observed in the field (5–20 nM, Figure 2a), assuming that N and P are biologically utilized at a N:P ratio of 16:1. There may be large uncertainties associated with these assumptions and calculations because the N_2 fixation rate used here is based on biomass estimation and assumes a constant N_2 fixation per unit biomass. A better understanding of the contribution of N_2 fixation in the SCS requires in situ measurements of the N_2 fixation rate in the field and the inclusion of other potential N_2-fixing microorganisms in the estimation. Besides *Trichodesmium*, the endosymbiont *Richelia intracellularis*, which is commonly observed within several marine diatoms species [*Carpenter et al.*, 1999], and unicellular cyanobacteria symbiont of heterotrophic marine dinoflagellates are potent in N_2 fixation and were not included in the present survey. Other N_2-fixing microorganisms with size smaller than 3 μm, which were detected by amplification of nitrogenase genes [*Zehr et al.*, 2001], are possible N_2-fixers.

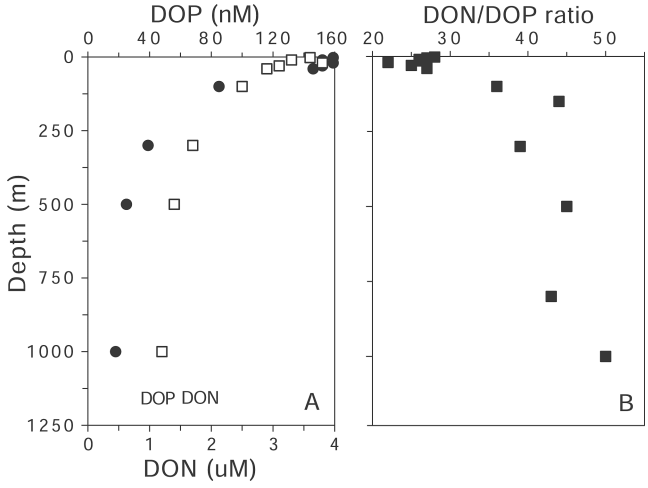


Figure 5. Vertical profiles of (a) DON and DOP and (b) DON:DOP ratio in the SCS.

These small N_2-fixers probably deserve more attention since >80% of phytoplankton biomass in the SCS is <3 μm.

[19] The low abundance of *Trichodesmium* and the indication of nitrogen limitation are unexpected because the SCS surface water conditions (warm, oligotrophic, permanently stratified, and dust/Fe rich) should favor the proliferation of N_2 fixing microorganism which would increase the supply of new N into the system thereby alleviating fixed-N limitation. So a key question remains: What limits N_2 fixation in the SCS?

[20] Important ecological factors that may limit the rate of N_2 fixation include: light intensity, temperature, as well as bioavailable P and Fe concentrations. In the stratified surface waters of the SCS, light and temperature should not be the limiting factors for N_2 fixation. These two parameters in the SCS are similar to those in the tropical central Atlantic where there was a high abundance of *Trichodesmium* [*Capone et al.*, 1997; *Sanudo-Wilhelmy et al.*, 2001]. Based on growth requirements of P by phytoplankton [*Geider and LaRoche*, 2002], we estimate a threshold of approximately 1.5 nM DIP, above which the orthophosphate diffusion can provide adequate phosphorus for the growth of a 8 μm diameter *Trichodesmium* cell growing at 0.08 d^{-1}. This DIP concentration is lower than what we observed in the SCS surface water by several-fold, suggesting that the growth of N_2 fixing microorganisms in the SCS are not limited by available phosphorus. The thrust of this argument rests with the reliability of *Trichodesmium* as a metabolic-model microorganism. The excess P supply from upward nutrient flux and in situ nutrient recycling supports the growth of microorganism like *Trichodesmium* which would be expected to supply new fixed N into surface ocean. The new N input into the surface water should lead to a biological depletion of surface water DIP. The lack of DIP depletion in the SCS (Figure 2) implies that N_2 fixation in the SCS may be controlled by other factors, perhaps Fe availability.

4.3. Fe Distribution

[21] Fe is a cofactor for enzyme nitrogenase [*Kustka et al.*, in press]. Organisms using N_2 as their sole source of nitrogen require one order of magnitude higher Fe than cells using fixed nitrogen [*Saino*, 1977; *Raven*, 1988; *Rueter*, 1983; *Rueter et al.*, 1992; *Paerl et al.*, 1994; *Sanudo-Wilhelmy et al.*, 2001]. Fe exists in seawater as particulate

Table 1. Occurrence ($\times 10^3$ trichomes m^{-3}) of Planktonic Nitrogen-Fixing Cyanobacteria *Trichodesmium* sp. and its Estimated Nitrogen Fixation Rate (μg N m^{-3} hr^{-1}) and Nitrate Uptake Rate (μg N m^{-3} hr^{-1}) in the South China Sea

Month	Depth, m	*Trichodesmium*	N_2-Fixation[a] Low	N_2-Fixation[a] High	NO_3 Uptake	N_2-Fixation/NO_3 Uptake, %
March 2000	2	16	0.03	0.24	13	0.23–1.85
March 2001	2	8	0.02	0.12	27	0.07–0.44
	13	1	<0.01	0.01	31	0–0.03
	34	1	<0.01	0.01	29	0–0.03
	51	19	0.04	0.28	21	0.19–1.33
	85	0	0	0	4	0
May 2001	2	8				
	5	13				
	10	27				
	20	18				
	40	4				
	50	18				
	60	3				
	80	7				
	100	1				
July 2001	2	48	0.09	0.72	18	0.50–4.00
	13	0	0	0	13	0
	16	10	0.02	0.15	11	0.18–1.05
	34	13	0.02	0.19	7	0.31–2.92
	51	0	0	0	5	0
	85	0	0	0	4	0

[a]Based on a fixing rate of 14.9 pg N trichome^{-1} hr^{-1} for *Trichodesmium spp.* [*Saino*, 1977] for high value, 1.9 pg N trichome^{-1} hr^{-1} [*McCarthy and Carpenter*, 1979] for low value.

(>0.4 μm) and dissolved (<0.4 μm) forms. Particulate Fe cannot diffuse easily in water and thus most of particulate Fe is not considered to be directly available to marine microorganisms. Dissolved Fe has long been considered to exist in seawater predominantly as soluble Fe-organic complexes that are available for assimilation [*Hutchins et al.*, 1999], although recent evidence has shown that only a small portion of the "dissolved" Fe exists as soluble Fe species whereas much of the "dissolved" Fe is present as less available colloidal particles of 0.02–0.4 μm diameter [*Wu et al.*, 2001]. The bioavailability of these pools is neither known nor easily determined.

[22] In the water column of the SCS in March 2000, "dissolved" (<0.4 μm) Fe concentrations increase from ~0.2 nM in the surface water to ~0.6 nM at depths below 500 m (Figure 6a). Particulate (>0.4 μm) Fe concentrations were low in the upper 100 m of the water column (~0.15 nM, Figure 6b). Below 100 m the particulate Fe concentrations increased to ~7 nM at 500 m and then decrease with increasing depth to 3 nM at 2500 m (Figure 6b). Particulate Fe concentrations in the surface water increased from 0.1–0.2 nM in March to 3–4 nM in July, whereas dissolved Fe concentrations increase from 0.2 nM in March to 0.3 nM in July. In July 2000, ~50% of "dissolved" Fe (~0.15 nM) was found in the colloidal fraction (i.e., 0.02–0.4 μm diameter). The SCS surface water dissolved Fe concentrations of 0.2–0.3 nM are similar to those in the central North Pacific near Hawaii (Figure 6a) [*Rue and Bruland*, 1995; *Wu et al.*, 2001], where N_2 fixation is considered to be limited by Fe, suggesting that in the SCS Fe availability may also limit the rate of N_2 fixation and thus indirectly control P inventories as well.

[23] The low concentrations of dissolved Fe are not expected for the dust-rich SCS [*Duce et al.*, 1991], because dissolved Fe concentrations in the stratified surface waters generally increase with increasing eolian Fe deposition [*Wu and Luther*, 1994; *Bruland et al.*, 1994; *Wu et al.*, 2001]. In the SCS, however, surface water dissolved Fe concentrations appear to be independent of dust deposition. As eolian Fe deposition accumulated in the surface mixed layer in July (as shown by 1 order of magnitude increase in particulate Fe at 20 m from 0.1–0.2 nM in March to 4 nM in July, Figure 6b), dissolved Fe concentrations increased from ~0.2 nM in March to 0.3 nM in July. The surface-minimum depth-enrichment dissolved Fe profile in the SCS is similar to those observed in dust-poor high nutrient low chlorophyll oceans where primary production is limited by low rate of eolian Fe deposition [*Martin and Gordon*, 1988]. These distributions are different from surface-maximum subsurface minimum dissolved Fe profiles typically observed in stratified oligotrophic oceans [*Bruland et al.*, 1994; *Wu and Luther*, 1994; *Wu et al.*, 2001]. We hypothesize that surface water dissolved Fe concentrations in the SCS may result from low Fe solubility due to a lack of Fe-binding organic ligands in the surface water.

[24] Inorganic Fe solubility in seawater (0.08 nM [*Wu et al.*, 2001]) is well below dissolved Fe concentrations that we observed in the SCS (0.2–0.3 nM, Figure 6a), suggesting that dissolved Fe in the SCS must exist, at least in part, as Fe-dissolved organic ligand complexes. Dissolved

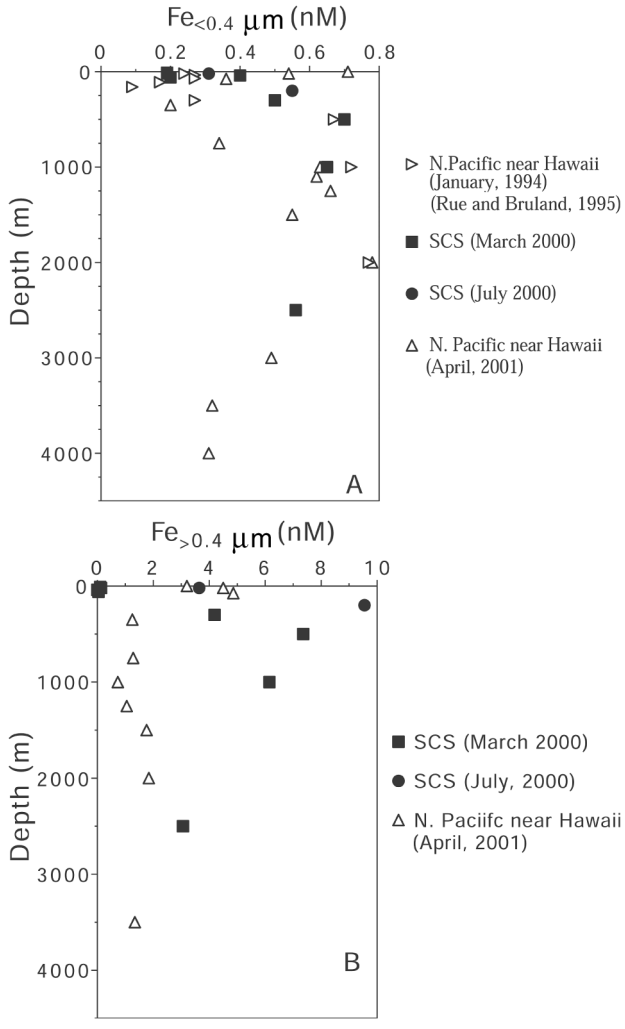


Figure 6. Vertical profiles of (a) dissolved and (b) particulate Fe in the SCS.

organic ligands capable of binding Fe in seawater have been observed in many ocean regions [*Rue and Bruland*, 1995; *Wu and Luther*, 1995; *van den Berg*, 1995; *Wu et al.*, 2001]. By forming dissolved Fe-ligand complexes, these ligands can increase seawater dissolved Fe concentrations above the inorganic Fe solubility limit. In the oligotrophic North Atlantic and North Pacific, ligand concentrations exceed total dissolved Fe [*Rue and Bruland*, 1995; *Wu et al.*, 2001] and additional eolian Fe can thus be dissolved in the water. Dissolved Fe concentrations in these waters increase with increasing eolian Fe deposition [*Wu and Boyle*, 2002]. In the SCS, however, dissolved Fe concentrations increase only slightly (from 0.2 to 0.3 nM) whereas particulate Fe concentrations increase much more substantially (probably due to eolian Fe deposition), suggesting that dissolved Fe concentration in the SCS may be controlled by a lack of excess organic ligands in the water. By decreasing the solubility of eolian Fe, the lack of Fe-binding organic ligands in the surface water may limit the concentrations of Fe that can be dissolved in the water. This hypothesis remains to be tested by direct electro-

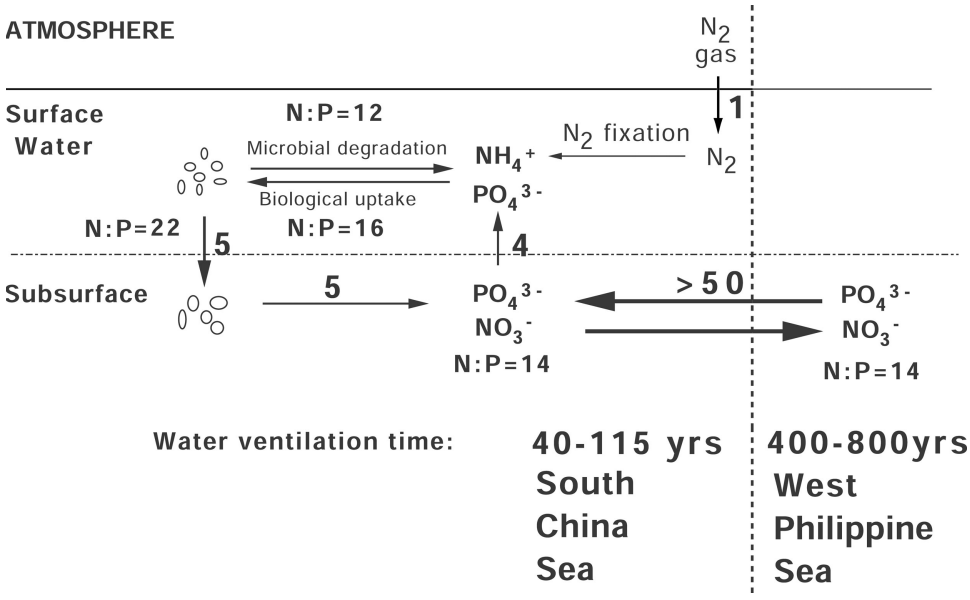


Figure 7. Nutrient cycles in the SCS.

chemical measurements of Fe-binding organic ligands in the SCS habitat.

[25] If Fe limits N_2 fixation in the SCS, can the increases in available Fe flux shift the SCS ecosystem from a N-limited to a P-limited state? The answer lies in the ability of N_2 fixing organisms to acquire P from semilabile or refractory dissolved organic P pools, from atmospheric deposition or from below the mixed layer. When the ecosystem is P-limited, surface water DIP will be assimilated until threshold levels below which uptake is no longer possible. At this point, if the N_2 fixing microbes can not obtain excess P from below the mixed layer, even if available Fe is ample, DIP limitation would decrease the rate of N_2 fixation in the surface water until the ecosystem eventually selects against N_2-fixing microorganisms by the remineralization of high N:P ratio organic matter. Over time, a selective export of N via particle sedimentation or denitrification causes fixed N limitation and the N_2-fixation cycle starts over again. However, if some portion of N_2 fixing microbial assemblage can assimilate excess P from P-rich subsurface waters through vertical migration [*Karl et al.*, 1992] or by utilizing a portion of the relatively large DOP pool (Bjorkman and Karl, in review), then the continued supply of new N from N_2 fixation in the presence of ample Fe will maintain the SCS ecosystem in long-term P-limitation status.

4.4. Nutrient Cycles

[26] A compelling picture emerges when the above referenced nutrient data are viewed in the context of nutrient cycle within the SCS water column and between the SCS and the WPS (Figure 7). In the SCS, water at the base of mixed layer has a DIP concentration of ~500 nM and a DNN concentration of 7000 nM (Figure 2). As this water is mixed to the sea surface, assimilation in a N:P molar ratio of 16:1 (Redfield ratio) should deplete N before P, leaving ~63 nM excess DIP in the surface water. The nutrient recycling through the microbial loop in the stratified surface water results in the production of high N:P ratio (28:1) dissolved organic matter (DOM) (Figure 5). The DOM export will leave an additional ~75 nM excess DIP in the surface water, if 20% of carbon export is via DOM. Although DOM export has been estimated to be 50% of the total at BATS and HOT [*Carlson et al.*, 1994; *Emerson et al.*, 1997], our calculation based on a simple mixing between the surface mixed layer (20 nM P, 160 nM DOP) and the base of the mixed layer (500 nM P and 80 nM DOP) suggests that DOM export may be only about 14% of total export in the SCS. The combination of two P sources leads to an excess DIP concentration of ~138 nM in the SCS surface water. We actually observed 5–20 nM DIP in the field (Figure 2), suggesting that new fixed N from N_2 fixation has been introduced to the surface water from N_2 fixation for biological depletion of the excess P. If the new N is fixed at a N:P ratio of 16:1 (Redfield ratio), the flux of new N supply by N_2 fixation (~1788 nM N) would be equal to ~25% of upward nitrate flux from the subsurface (~7000 nM N). Thus, for every four atoms of fixed N upwelling from the subsurface, there is one atom of new N supplied by N_2 fixation (Figure 7). At this rate, the new N input from N_2 fixation appears to play a minor role in altering the subsurface water N:P ratio. The similarity in subsurface water nutrient concentrations and N:P ratio between the SCS and the WPS (Figure 3) suggests that water exchange between the two seas may have erased the excess N signal coming from N_2 fixation, or the system may be balanced by water column denitrification by water mixing through Luzon Strait. Our data suggest that the water exchange between the two seas are so active that it does not allow the extra N from N_2 fixation to accumulate in the SCS subsurface water.

[27] Based on the low rate of N_2 fixation in the SCS surface waters that contains low concentrations of DIP, we hypothesize that the upward flux of available P from the base of the mixed layer may be negligible compared to nutrient flux through the LS strait. In the present-day oligotrophic SCS surface water, steady state DIP concentrations are ~5–20 nM (Figure 2). Alleviating Fe limitation

of N_2 fixation can only increase the N_2 fixation rate ~15% over the current rate which is equivalent to the depletion of ~138 nM excess DIP (see above discussion) before DIP is completely consumed biologically. A further increase of new N input by N_2 fixation requires an increase in upward mixing of P-rich subsurface water or an increase in the N:P ratio of DOM exported from the surface mixed layer, which in turn are controlled by upper water column stratification and resulting changes in microbial loop activities in the surface water. It can be expected that during past geological time period (e.g., the Last Glacial Maximum) when the SCS upper water column was less stratified and the atmospheric dust loading (and thus the available Fe input) was higher, a substantial increase in surface water N_2 fixation rate would increase subsurface water N:P ratio to over the Redfield N:P stoichiometry of 16:1, and lead to a shift from current state of N-limitation to P-limitation.

5. Conclusion

[28] The euphotic zone in the SCS basin is deficient in nitrogen, phosphorus and Fe, but with a larger relative N depletion compared to cell needs. This assertion is supported by the observations of low DNN/DIP, relatively high DIP concentration and phytoplankton proliferation response to nitrate enrichment, but not to phosphate enrichment. The degree of N deficit is possibly related to the restrained growth of N_2-fixing microorganisms such as *Trichodesmium* spp. *Trichodesmium* is sparsely distributed in the SCS and the estimated contribution of N_2 fixation to the new nitrogen budget for phytoplankton growth at present is far smaller than that from the nitrate input from vertical mixing. The insignificance of N_2-fixation in this oligotrophic ocean may result from a limited availability of iron, thereby limiting N_2 fixation. We hypothesize that the lack of iron-binding organic ligands in the SCS limits iron solubility and bioavailable iron concentrations, and consequently restrains the growth of *Trichodesmium*, and other N_2-fixing microorganisms, in this region despite relatively high iron input.

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References

Abell, J., S. Emerson, and P. Renaud, Distributions of TOP, TON and TOC in the North Pacific subtropical gyre: Implications for nutrient supply in the surface ocean and remineralization in the upper thermocline, *J. Mar. Res.*, *58*, 203–222, 2000.

Armstrong, F. A. J., and S. Tibbets, Photochemical combustion of organic matter in sea water, for nitrogen, phosphorus, and carbon determination, *J. Mar. Biol. Assoc. U.K.*, *48*, 143–152, 1968.

Bell, J., J. Betts, and E. A. Boyle, MITESS: A moored in-situ trace element serial sampler for deep-sea moorings, *Deep Sea Res.*, in press, 2002.

Broecker, W. S., W. C. Patzert, J. R. Toggweiler, and M. Stuiver, Hydrography, chemistry, and radioisotopes in the southeast Asian basins, *J. Geophys. Res.*, *91*, 14,345–14,354, 1986.

Bruland, K. W., K. J. Orians, and J. P. Cowen, Reactive trace metals in the stratified central North Pacific, *Geochim. Cosmochim. Acta*, *58*, 3171–3182, 1994.

Capone, D. G., J. P. Zehr, H. W. Pearl, B. Bergman, and E. J. Carpenter, *Trichodesmium*, a globally significant marine cyanobacterium, *Science*, *276*, 1221–1229, 1997.

Carlson, C. A., H. W. Ducklow, and A. F. Michaels, Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea, *Nature*, *371*, 405–408, 1994.

Carpenter, E. J., and C. C. Price, IV, Marine *Oscillatoria* (*Trichodesmium*): Explanation for aerobic nitrogen fixation without heterocysts, *Science*, *191*, 1278–1280, 1976.

Carpenter, E. J., J. P. Montoya, J. Burns, M. R. Mulholland, A. Subramaniam, and D. G. Capone, Extensive bloom of a N_2-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean, *Mar. Ecol. Prog. Ser.*, *185*, 273–283, 1999.

Chang, J., K. Chiang, and G. Gong, Seasonal variation and cross-shelf distribution of the nitrogen-fixing cyanobacterium, *Trichodesmium*, in southern East China Sea, *Cont. Shelf Res.*, *20*, 479–492, 2000.

Chen, Y. L., H. Lu, F. Shiah, G. Gong, K. Liu, and J. Kanda, New production and f-ratio on the continental shelf of the East China Sea: comparisons between nitrate inputs from the subsurface Kuroshio Current and the Changjiang River, *Estuarine Coastal Shelf Sci.*, *48*, 59–75, 1999.

Codispoti, L. A., Phosphorus vs. nitrogen limitation in new and export production, in *Productivity of the Oceans: Present and Past*, edited by W. H. Berger, V. S. Smetacek, and G. Wefer, pp. 377–394, John Wiley, New York, 1989.

Deutsch, C., N. Gruber, R. M. Key, J. L. Sarmiento, and A. Ganachaud, Denitrification and N_2 fixation in the Pacific Ocean, *Global Biogeochem. Cycles*, *15*, 483–506, 2001.

Dickey, T., et al., Preliminary results from the Bermuda testbed mooring program, *Deep Sea Res., Part I*, *45*, 771–794, 1998.

Duce, R. A., et al., The atmospheric input of trace species to the world ocean, *Global Biogeochem. Cycles*, *5*, 193–259, 1991.

Dugdale, R. C., and F. P. Wilkerson, The use of ^{15}N to measure nitrogen uptake in eutrophic oceans: Experimental considerations, *Limnol. Oceanogr.*, *31*, 673–689, 1986.

Emerson, S., P. Quay, D. M. Karl, C. Winn, L. Tupas, and M. Landry, Experimental determination of the organic carbon flux from open ocean surface waters, *Nature*, *389*, 951–954, 1997.

Falkowski, P. G., Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean, *Nature*, *387*, 272–275, 1997.

Garside, C., A chemiluminescent technique for the determination of nanomolar concentrations of nitrate and nitrite in seawater, *Mar. Chem.*, *11*, 159–167, 1982.

Geider, R. J., and J. LaRoche, Redfield revisited: Variability of CNP in marine microalgae and its biochemical basis, *Eur. J. Phycol.*, *37*, 1–17, 2002.

Gong, G. C., K. K. Liu, C. T. Liu, and S. C. Pai, The chemical hydrography of the South China Sea west of Luzon and a comparison with the West Philippine Sea, *Terr. Atmos. Oceanic Sci.*, *3*, 587–602, 1992.

Hutchins, D. A., A. E. Witter, A. Butler, and G. W. Luther III, Competition among marine phytoplankton for different chelated iron species, *Nature*, *400*, 858–861, 1999.

Jackson, G. A., and P. M. Williams, Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling, *Deep Sea Res.*, *32*, 223–235, 1985.

Karl, D. M., and G. Tien, MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments, *Limnol. Oceanogr.*, *37*, 105–116, 1992.

Karl, D. M., R. Letelier, D. V. Hebel, D. F. Bird, and C. D. Winn, *Trichodesmium* blooms and new production in the North Pacific gyre, in *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*, edited by E. J. Carpenter, pp. 219–237, Kluwer Acad., Norwell. Mass., 1992.

Karl, D. M., R. Letelier, L. Tupas, J. Dore, J. Christian, and D. V. Hebel, The role of nitrogen fixation in the biogeochemical cycling in the subtropical North Pacific Ocean, *Nature*, *388*, 533–538, 1997.

Karl, D. M., A. Michaels, B. Bergman, D. Capone, E. Carpenter, R. Letelier, F. Lipschultz, H. Paerl, D. Sigman, and L. Stal, Dinitrogen fixation in the world's oceans, *Biogeochemistry*, *57/58*, 47–98, 2002.

Kustka, A., S. Sañudo-Wilhelmy, E. J. Carpenter, D. G. Capone, and J. A. Raven, A revised estimate of the Fe use efficiency of nitrogen fixation, with special reference to the marine N_2 fixing cynobacterium, *Trichodesmium spp.* (Cyanophyta), *J. Phycol.*, in press, 2002.

Letelier, R. M., and D. M. Karl, *Trichodesmium* spp. physiology and nutrient fluxes in the North Pacific subtropical gyre, *Aquat. Microbiol. Ecol.*, *15*, 265–276, 1998.

Martin, J. H., and R. M. Gordon, Northeast Pacific iron distributions in relation to phytoplankton productivity, *Deep Sea Res.*, *35*, 177–196, 1988.

McCarthy, J. J., and E. J. Carpenter, Oscillatoria (Trichodesmium thiebautii (Cyanophyta) in the central North Atlantic Ocean, *J. Phycol.*, *15*, 75–82, 1979.

Paerl, H. W., L. E. Prufert-Bebout, and C. Guo, Iron stimulated N_2 fixation and growth in natural and cultured populations of the planktonic marine cyanobacteria *Trichodesmium spp*, *Appl. Environ. Microbiol.*, *60*, 1044–1047, 1994.

Raven, J. A., The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources, *New Phytol.*, *109*, 279–287, 1988.

Rue, E. L., and K. W. Bruland, Complexation of iron(III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method, *Mar. Chem.*, *50*, 117–139, 1995.

Rueter, J. G., Theoretical iron limitation of microbial N_2 fixation in the oceans, *Eos Trans. AGU*, *64*, 445, 1983.

Rueter, J. G., D. A. Hutchins, R. W. Smith, and N. L. Unsworth, Iron nutrition of *Trichodesmium*, in *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*, edited by E. J. Carpenter, D. G. Capone and J. G. Rueter, pp. 289–306, Kluwer Acad., Norwell, Mass., 1992.

Ryther, J. G., and W. M. Dunstan, Nitrogen, phosphorus, and eutrophication in the coastal marine environment, *Science*, *171*, 1008–1013, 1972.

Saino, T., Biological nitrogen fixation in the ocean with emphasis on the nitrogen fixing blue-green alga Trichodesmium and its significance in the nitrogen cycling in the low latitude sea areas, Ph. D. thesis, Univ. of Tokyo, Tokyo, 1977.

Sanudo-Wilhelmy, S. A., A. B. Kustka, C. J. Gobler, D. A. Hutchins, M. Yang, K. Lwiza, J. Burns, D. G. Capone, J. A. Raven, and E. J. Carpenter, Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean, *Nature*, *411*, 66–69, 2001.

Shaw, P. T., The seasonal variation of the intrusion of the Philippine Sea water into the South China Sea, *J. Geophys. Res.*, *96*, 821–827, 1991.

Strickland, J. D. H., and T. R. Parsons, A practical handbook of seawater analysis, *Bull. Fish. Res. Board Can.*, *167*, 1–310, 1972.

van den Berg, C. M., Evidence for organic complexation of iron in seawater, *Mar. Chem.*, *50*, 139–157, 1995.

Wang, J., Observation of abyssal flows in the northern South China Sea, *Acta Oceanogr. Taiwan.*, *16*, 36–45, 1986.

Wu, J., and E. A. Boyle, Determination of iron in seawater by high resolution isotope dilution inductively coupled plasma mass spectrometry after $Mg(OH)_2$ coprecipitation, *Anal. Chim. Acta*, *367*, 183–191, 1998.

Wu, J., and E. A. Boyle, Iron in the Sargasso Sea: Implications for the processes controlling dissolved iron distribution in the ocean, *Global Biogeochem. Cycles*, *16*, 1086, 10.1029/2001GB001453, 2002.

Wu, J., and G. W. Luther III, Size fractionated iron concentrations in the water column of the western north Atlantic Ocean, *Limnol. Oceanogr.*, *39*, 1119–1129, 1994.

Wu, J., and G. W. Luther III, Complexation of Fe(III) by natural organic ligands in the northwest Atlantic Ocean by competitive ligand equilibration method and kinetic approach, *Mar. Chem.*, *50*, 159–177, 1995.

Wu, J., W. Sunda, E. A. Boyle, and D. M. Karl, Phosphate depletion in the Western North Atlantic Ocean, *Science*, *289*, 759–762, 2000.

Wu, J., E. A. Boyle, W. Sunda, and L. S. Wen, Soluble and colloidal iron in oligotrophic oceans, *Science*, *293*, 847–849, 2001.

Zehr, J. P., et al., Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean, *Nature*, *412*, 635–638, 2001.

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