Physical-biological coupling of N₂ fixation in the northwestern South China Sea coastal upwelling during summer

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Abstract

Here, we present the first combined results of N₂ fixation rates ($^{15}N_2$ assay), dissolved iron (dFe, < 0.2 μ m), and primary production (PP) (¹⁴C assay) in the northwestern South China Sea (NWSCS) coastal upwelling region during summer. Surface N₂ fixation rate ranged between 0.1 nmol N L⁻¹ d⁻¹ and 5.6 nmol N L⁻¹ d⁻¹ (average 1.0 nmol N L⁻¹ d⁻¹, n = 50) under nonbloom conditions. At a *Trichodesmium* bloom station, N₂ fixation rate was \sim 3 orders of magnitude higher. Depth-integrated N₂ fixation rate ranged between 7.5 μ mol N m⁻² d⁻¹and 163.1 μ mol N m⁻² d⁻¹ (average 46.4 μ mol N m⁻² d⁻¹). Our results indicate that N₂ fixation is unlikely limited by Fe availability in the NWSCS continental waters, instead, the coastal upwelling-induced combined effects of physical and biological processes may have played a decisive role. With the upwelled cold, dFe-rich, nutrient-replete waters, nondiazotrophic phytoplankton growth would be preferentially enhanced while N₂ fixation was hindered due to relative deficiency of phosphate caused by massive phytoplankton utilization in the coastal upwelling. By comparison, N₂ fixation was notably elevated along with decreased PP in the offshore waters, probably due to a shift from P-deficiency to N-deficiency. Consistently, the contribution of N_2 fixation to PP (0.01–2.52%) also increased toward the open waters. As a significant external N source, summertime N₂ fixation is estimated to contribute a flux of 1.4 Gmol N to this area under nonbloom conditions. This study adds to the knowledge of N2 fixation in the rarely studied subtropical coastal upwellings, and highlights the necessity of future comprehensive studies in such highly dynamic environments.

Nitrogen fixation plays an important role in marine biogeochemical cycles, as it adds fixed N to the surface ocean and regulates net sequestration of atmospheric CO₂, thus exerting profound impacts on global climate (Karl et al. 2002; Capone et al. 2005). Until recently, most of the attentions has been paid to the oligotrophic tropical and subtropical open ocean waters. In contrast, coastal upwellings, which play a disproportionately important role in the cycling of marine nutrients (Capone and Hutchins 2013), have long been ignored in respect of N2 fixation. It may be partly because some environmental conditions (such as relatively low seawater temperature) in upwellings are traditionally considered to be not favorable for N2 fixation. However, there is growing evidence from field observations that N2 fixation may also be actively occurring in the tropical and subtropical upwelling regimes. To date, there are few reported

N₂ fixation studies in limited upwelling regimes in global ocean, including the Benguela upwelling (Sohm et al. 2011a), Equatorial upwelling (Subramaniam et al. 2013), and Vietnamese upwelling in the southern South China Sea (SCS) (Voss et al. 2006). It is notable that the knowledge of N₂ fixation in coastal upwellings is very lacking, which in turn may substantially weaken our efforts to better understand marine N cycle for both present and past.

The SCS is the largest marginal sea in the western Pacific. It has wide continental shelves in the northwest and south and a deep basin with a depth of 4700 m. Extending from the equator to 22°N, its western boundary is a broad shelf off the coast of mainland China and Vietnam and generally shallower than 100 m. The general circulation in the SCS, including the waters around Hainan Island, is susceptible to monsoons, which prevails all year round with the northeasterly winds in winter and southwesterly winds in summer (Chai et al. 2001). The northwestern South China Sea

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(NWSCS) is very dynamic with respect to hydrological conditions, where coastal upwelling around Hainan Island is one most prominent feature on the continental shelf during summer (see more in "Sampling" part). Thus, it is reasonable to speculate that such dynamic physical oceanographic conditions should exert impacts on marine biogeochemical processes profoundly, including N2 fixation. Unfortunately, direct field measurements of N₂ fixation rate in the whole SCS are few to date. The few published studies reported directly measured N₂ fixation rate are generally confined to the SCS deep basin (Chen et al. 2008, 2014; Zhang et al. 2011) and southern SCS off Vietnam coast (Voss et al. 2006; Grosse et al. 2010). Interestingly, the work of Sohm et al. (2011a) had suggested that N₂ fixation may be much more prevalent in the upwelling regimes than previously recognized. However, no direct measurements of N2 fixation rate have been reported in the NWSCS, which is highly impacted by coastal upwelling.

Coastal upwellings may act as a key physical driving force on nutrient and phytoplankton dynamics (Capone and Hutchins 2013), which will likely exert a profound impact on N₂ fixation. For example, iron has been proposed as a key limiting micronutrient for N2 fixation in most open ocean waters globally (Karl et al. 2002; Sohm et al. 2011c), while its relation to N₂ fixation in coastal upwellings generally remains unknown due to lack of samplings. In fact, no concurrent measurements of N2 fixation rate and dissolved iron concentration (dFe) in the whole SCS have been reported, while such reports are also few for the global ocean (Sohm et al. 2011c; references therein). It has been proposed that relatively low Trichodesmium abundance may be mainly induced by dFe deficiency in the SCS basin, while the lack of organic ligands has been ascribed as a major cause even though the SCS receives some of the largest fluxes of atmospheric dust among the world oceans (Wu et al. 2003). It has been reported that upwellings play a decisive role in regulating dFe pattern in coastal upwelling regions (Bruland et al. 2001). The possible influence of iron on N₂ fixation in the SCS coastal upwelling regions should be examined based on concurrent direct measurements. Besides, along with their highly variable physical oceanography, coastal upwellings may also play a significant role in regulating phytoplankton community structure and the subsequent utilization of macronutrients, which will finally exert significant influence on N₂ fixation (Church et al. 2009; Mills and Arrigo 2010). Unfortunately, knowledge on these aspects in coastal upwellings still remains insufficient, while no such study has been conducted in the NWSCS.

One central goal of this study is to examine whether N_2 fixation can be important in the upwelling regimes in the NWSCS, as reported in the Benguela Upwelling of the South Atlantic unexpectedly (Sohm et al. 2011*a*). We aim to obtain the spatial pattern of N_2 fixation rate in the rarely studied subtropical NWSCS, which is largely influenced by coastal

upwelling during summer. N2 fixation and primary production (PP) rates were measured using isotope tracer assay (¹⁵N₂ and ¹⁴C-HCO₃⁻, respectively). We will also measure concentration of dissolved iron (dFe) to evaluate how iron may affect N₂ fixation in the study area. PP will be measured to examine whether there is a coupling between N₂ fixation and nondiazotrophic carbon fixation or not. Finally, we will estimate the N₂ fixation flux during summer for the NWSCS continental shelf, as such information is still blank in this area but is of significant implications. The abundance of the globally important filamentous diazotrophic cyanobacteria, Trichodesmium, will also be measured. Several studies in the subtropical areas have observed that Trichodesmium was dominant near islands (e.g., Hawaiian Islands) or continent (e.g., Vietnam coast), while the unicellular (< 10 μ m) diazotrophs dominate in the areas remote from the islands (Moisander et al. 2008; Sohm et al. 2011b). We will try to explain the observed N₂ fixation variability from a perspective of physical-biological coupling. In addition, the contribution of N₂ fixation as an external N source in the NWSCS during summer will be evaluated.

Sampling

Background of upwelling around Hainan Island

Coastal upwelling around Hainan Island is one most prominent feature of physical oceanography in the NWSCS during summer. Although the coastal waters west and east off the Hainan Island are both characterized by upwelling during summer, the mechanisms are different. The upwelling off the east coast and northeast of Hainan (also known as the Qiongdong upwelling and Leizhou Peninsula upwelling) has been recognized since 1960s, and is mainly attributed to the combined results of monsoon, topography, and coastline shape (Chai et al. 2001; Jing et al. 2011). It reaches maximum in summer when the southwesterly monsoon prevails. By comparison, the causes for the summertime upwelling west off Hainan Island are somewhat more complex and has not been recognized until recently. At first glance, such upwelling seems unlikely as the direction of the summer prevailing winds is generally parallel to the west coastline of Hainan Island and should result in coastal downwelling. However, due to the existence of a strong tidal mixing front, the net effect is weak upwelling (Lü et al. 2008). The region under the influence of coastal upwelling may be much broader than the band of active upwelling, because the recently upwelled water can be transported far offshore toward the open waters (Chai et al. 2001; Lü et al. 2008; Jing et al. 2011).

Mean offshore velocity from ADCP

To verify the occurrence of coastal upwelling (evidenced by offshore flow in the upper water column and onshore flow below) east off Hainan Island, an upward looking Acoustic Doppler Current Profiler (ADCP, RDI 300 kHz, Workhorse Sentinel) was moored on seafloor at 110.48°E,



Fig. 1. Sampling locations for N₂ fixation in the northwestern South China Sea (SCS). The inset shows the SCS. The stations with prefix "D" were sampled during "Hainan-E" cruise (n = 32), while the stations with prefix "H" or "J" were sampled during "Hainan-W" cruise (n = 19), respectively. The black dots represent stations where N₂ fixation was sampled only for surface (~ 1 m) waters. The blue triangles represent stations where depth profiles of N₂ fixation were sampled (n = 11). The yellow square denotes Sta. D15-12, where a *Trichodesmium* spp. bloom was encountered. The mouth of Pearl River (Zhujiang) is denoted by "ZJ" in the inset map.

18.60°N (station water depth of 82 m) east off Hainan Island during 25 June–26 July 2006. It was sampled using 2 m vertical bins and 10 min averages. The along shore velocity was taken as "v" component, and the cross direction as "u" component. Finally, the time averaged u and v are used. More details of data processing can be found in Emery and Thomson (2001).

Seawater sample collection

Sampling stations for seawater collections in the NWSCS (17-22°N, 108-114°E) in 2006 summer are shown in Fig. 1. The stations with prefix "D" (n = 32) were sampled in the cruise (Hainan-E) during 19 July-06 August onboard R/V SHIYANSANHAO, while the stations with prefix "H" or "J" (n = 19) were sampled on another cruise (Hainan-W) during 30 July-07 August onboard R/V SHIYANERHAO, respectively. A total of 50 stations were visited and most are located on the continental shelf with water depth < 200 m. The sampling covered part of the Beibu (Tonkin) Gulf. Seawater samples were collected from standard depths (0 m, 10 m, 30 m, 100 m) for N₂ fixation, carbon fixation, and Trichodesmium abundance measurements using 10 L Niskin bottles mounted on a SeaBird 911 conductivity, temperature, and depth (CTD) rosette or acid-cleaned (0.1 N HCl) plastic bucket (for surface sampling stations).

Physico-chemical parameters

Seawater temperature and salinity were recorded by CTD sensors during the casts. Nitrate and phosphate concentrations were measured using a visible spectrophotometer after the standard colorimetric methods while onboard (Kirkwood et al. 1996). The detection limits for nitrate, phosphate, and silicate were 0.05 μ mol L⁻¹, 0.02 μ mol L⁻¹, and 0.45 μ mol L⁻¹, respectively. Nutrient data for Hainan-W cruise are not available due to technical reasons. Distribution of sea surface temperature (SST), wind, and chlorophyll were also obtained from satellite remote sensing for better understanding the impact of coastal upwelling. SST and wind data were obtained from National Oceanic and Atmospheric Administration (NOAA) Climatic Data Center (http://www.ncdc. noaa.gov/). Moderate Resolution Imaging Spectroradiometer (MODIS) chlorophyll data were obtained from the Distributed Active Archive Center (http://nssdc.gsfc.nasa.gov/earth/daacs.html) of NASA.

N₂ fixation rate

 N_2 fixation rates were measured for surface (~ 1 m) water at 50 stations, of which 11 stations were sampled for vertical profiles (< 100 m). $^{15}N_2$ tracer assay was applied for measuring N_2 fixation rate (Montoya et al. 1996). Briefly, duplicate seawater samples were filled bubble-free into cleaned (soaked in 0.1 N HCl for 24 h and washed with Milli-Q water) 600 mL clear borosilicate glass bottles and sealed, then spiked with 1.0 mL ^{15}N -labeled N_2 (99 atom% ^{15}N) via a septum using a gastight syringe (VICI), with the pressure across the septum being balanced by another syringe. The bottles were gently shaken for ~ 100 times before being placed in a deck incubator for 24 h with flowing seawater pumped from sea surface under natural (1 m: 100%) or simulated light levels (10 m: 50%; 30 m: 10%,; bottom or 100 m: 1%, using light screens), as estimated using a Secchi disk (mean 1% surface light euphotic zone depth was 53 m).

 N_2 fixation samples were gently filtered (<100 mm Hg) onto precombusted (450°C, 4 h) Whatman GF/F membranes, then dried (60°C) and stored frozen (-20°C) while on the sea. Natural suspended particulate organic matter samples were also collected for ¹⁵N analysis by filtering ~ 4 L seawater onto GF/F filters. Before isotopic analysis, the filters were dried (60°C, 24 h) again. Particulate organic nitrogen (PON) concentration and its ¹⁵N abundance on the filters were measured on a Finnigan Delta Plus XP isotope ratio mass spectrometer interfaced with an elemental analyzer (Carlo Erba NC 2500). The reproducibility for δ^{15} N measurements was better than 0.2‰. N₂ fixation rate (NFR) is calculated as follow:

$$NFR(nmol \ N \ L^{-1} \ d^{-1}) = \frac{1}{\Delta t} \times \left(\frac{A_{PON_f} - A_{PON_0}}{A_{N_2} - A_{PON_0}}\right) \times \frac{PON_0 + PON_f}{2}$$

where *t* is the incubation duration (1 d), PON₀ and PON_f is the concentration of PON at t = 0 and incubation ending, and A_{PON_f} , A_{PON_0} , and A_{N2} are absolute abundance of ¹⁵N in PON₀, PON_f, and N₂ pools, respectively. N₂ solubility in seawater is calculated after Weiss (1970). Areal rates of N₂ fixation (μ mol N m⁻² d⁻¹) were calculated with trapezoidal integration method.

Primary production

PP was measured using ¹⁴C tracer assay (Wolfe and Schelske 1967) for surface water ($\sim 1 \text{ m}$) at 49 stations (Sta. H17 not sampled), of which six stations (J57, J59, J61, J82, H12, H17) were sampled for vertical profiles. Briefly, seawater samples (100 mL) collected were immediately transferred into three acid-washed 125 mL glass bottles, two assigned as light, and one as dark. 0.8 μ Ci NaH¹⁴CO₃ was added to each bottle. The bottles for incubation were fitted with appropriate screens to simulate the light densities as described above and placed in deck incubator with flowing surface seawater. After 24 h incubation, PP samples were gently (< 100 mm Hg) filtered onto mixed cellulose ester membranes (pore size 0.2 μ m), before being stored frozen (-20°C). Inorganic carbon on the sample filters was removed by acid fuming. The assimilated ¹⁴C radioactivity was measured on a liquid scintillation counter (Perkin-Elmer TriCarb 2900TR). PP rates were calculated as:

$$PP(\mu mol \ C \ L^{-1} \ d^{-1}) = \frac{(R_S - R_B) \times TCO_2}{R \times \Delta t}$$

where $R_{\rm S}$ and $R_{\rm B}$ are the radioactivities of ${}^{14}{\rm C}$ ($\mu{\rm Ci}$) in light and dark bottles after correction for quenching, respectively, R is the added radioactivity of NaH¹⁴CO₃, and TCO₂ is the total carbon dioxide ($\mu{\rm mol} \ {\rm C} \ {\rm L}^{-1}$) in seawater. Depthintegrated PP (mmol C $m^{-2} d^{-1}$) was calculated also after trapezoidal integration.

Trichodesmium analysis

Trichodesmium abundance in surface water was analyzed at 35 stations. Vertical samples were collected at four (H17, H14, H12, J82) of the stations south of Hainan Island. Briefly, 1 L seawater samples collected using clean plastic bucket or CTD sampler were immediately transferred to high density polyethylene (HDPE) bottles and fixed with 10 mL acid (with acetic acid) Lugol's solution. While in land laboratory, subsamples were settled for 48 h before removing the supernatant. *Trichodesmium* was then identified and counted on a Nikon Eclipse 50i optical microscope. Number of *Trichodesmium* trichomes was converted to natural abundance in seawater (trichomes L⁻¹).

Concentration of dissolved iron (dFe)

Surface seawater samples for measuring concentration of dissolved iron (dFe) were collected at 42 stations. Rigorous clean sampling procedures were used throughout the sample collection and processing (Wu 2007). Nalgene HDPE and Teflon bottles were used for sampling, storage, and sample processing. Sampling bottles were attached to a bamboo rod (\sim 10 m long) with polypropylene holder and lowered to ~ 0.5 m below sea surface (soaked for several minutes) from the bow of the ship as soon as the ship was approaching (speed \sim 3 knots) the sampling locations. Once filled and sealed, the bottles were carefully transported to and filtered through acid-cleaned polycarbonate membranes (pore size of 0.2 µm, Millipore) mounted on 47 mm-diameter Teflon filter holders inside an over-pressurized class-100 clean air van. The filtrates were stored in Teflon bottles. The filtrate samples (30 mL collected each bottle) were acidified with 60 μ L 6 N HCl (Fisher Scientific Optima grade, purified with a quartz still) and stored at room temperature. Concentrations of dissolved iron was measured on a high resolution inductively coupled plasma mass spectrometry (FinniganTM ELEMENT2) in Jingfeng Wu's Lab in University of Alaska, Fairbanks, after the ⁵⁷Fe isotope dilution approach (Wu 2007). The procedure blank of dFe experiment was measured as 0.13 nM.

Results

Physico-chemical conditions

The occurrence of upwelling is clearly evidenced by the ADCP derived mean velocity profile (Fig. 2). The surface offshore velocity is much larger ($\sim 35 \text{ cm s}^{-1}$). The onshore flow occurred at 34 m and then increased with depth, with the maximum at 44 m. It then generally decreased downward to about zero.

Horizontal distributions of in situ measured seawater temperature, salinity, and nutrient (nitrate, phosphate) concentrations in surface (~ 1 m) and 30 m waters were shown in Fig. 3. SST ranged between 25.26°C and 30.69°C. Sea surface



Fig. 2. Vertical profile of mean offshore velocity derived from mooring ADCP. The cross in the inset indicates the ADCP mooring station (MS, water depth of 82 m) in the inset map. It was sampled between 25 June and 26 July 2006. Arrows with positive value mean offshore velocity. The shaded area represents seafloor.

salinity (SSS) ranged between 33.63 and 34.53. As expected, much lower SST values were observed in the east/northeast/ west off Hainan Island, in accordance with the occurrence of coastal upwelling. It is noted that the coastal waters near the Qiongzhou Strait have somewhat lowered SSS (~ 33.50). The 30 m waters are characterized by much lower temperature but higher salinity, also pointing to the occurrence of upwelling. Surface water [NO₃] ranged between 0.16 μ mol L^{-1} and 0.92 μ mol L^{-1} for our sampling stations. Phosphate was generally undetectable except for the coastal Sta. D14-5, where $[PO_4^{3-}]$ was slightly above the detection limit (0.02) μ mol L⁻¹). Similarly, much elevated nitrate (~ 10 μ mol L⁻¹) and phosphate concentrations (~ 0.6 μ mol L⁻¹) were observed in 30 m for the coastal stations. Distribution patterns of physico-chemical parameters (temperature, salinity, potential density, and nutrients) along D18 transect are shown in Fig. 4. Such depth profile also pointed to existence of upwelling. Monthly remote sensing SST data show a similar pattern to that of in situ measured SST (Fig. 5A). Monthly satellite remote sensing image shows that chlorophyll concentrations were generally low (< 0.15 μ g L⁻¹) in the offshore open waters, indicating low PP, which is quite different from the coastal upwelling waters (Fig. 5B).

Concentration of dissolved Fe (dFe)

dFe concentration fell in a range of 1.8-43.2 nmol L^{-1} (8.4 ± 9.6 nmol L^{-1}). Much higher values were encountered at the coastal stations relative to the offshore stations (Fig. 6). Values of dFe concentrations observed in this study are comparable with those reported in the surface waters of the adjacent southern East China Sea (ECS) coastal upwelling



Fig. 3. Horizontal distributions of seawater temperature, salinity, nitrate concentration, and phosphate concentration in 1-m and 30-m waters. Depth = 1 m: A, C, E, and G for temperature, salinity, nitrate concentration, and phosphate concentration; depth = 30 m: B, D, F, and H for temperature, salinity, nitrate concentration, and phosphate concentration, respectively. Temperature and nutrient concentrations are in units of °C and µmol L⁻¹, respectively. Note that more hydrological stations for temperature, salinity, and nutrient concentrations than N₂ fixation study were sampled during the cruise. Data were provided by Chinese Offshore Investigation and Assessment program.

(Jiann and Wen 2012), but an order of magnitude higher than those ($\sim 0.3 \text{ nmol L}^{-1}$) measured in the SCS basin (Wu et al. 2003; Wen et al. 2006).

Trichodesmium abundance

Trichodesmium was detected in surface waters at 34 of 35 stations. Interestingly, a high surface accumulation of *Trichodesmium* was encountered at Sta. D15-12 (19.23°N, 112.05°E), where *Trichodesmium* reached a surface density of 2797



Fig. 4. Depth profiles of seawater (A) temperature, (B) salinity, (C) potential density (σ_{θ}), (D) nitrate concentration, (E) phosphate concentration, and (F) silicate concentration along D18 transect. The units for temperature and all nutrient concentrations are °C and μ mol L⁻¹, respectively.

trichomes L^{-1} , indicating a bloom condition. This is the first time for such high diazotroph density to be reported in the NWSCS to our knowledge. As for the whole SCS, reports of

diazotrophic blooms are also few (Li et al. 2008). The onset of this *Trichodesmium* bloom is unclear and we did not know the spatial extension, either. Under nonbloom condition,



Fig. 5. Satellite remote sensing monthly mean sea surface (A) temperature superimposed by mean wind vectors and (B) chlorophyll concentration. Temperature, wind vector, and chlorophyll concentration are in units of °C, m s⁻¹ and μ g L⁻¹, respectively. Data of SST and wind are from NOAA National Climatic Data Center (NCDC). Data of chlorophyll are from the Distributed Active Archive Center (DAAC) of NASA. The spatial resolution is 4 × 4 km. Note that the chlorophyll data are shown on a log scale.



Fig. 6. Concentration of dissolved iron (dFe in unit nmol L^{-1}) in surface ($\sim 0.5~m$) water.

surface *Trichodesmium* abundance ranged between 0 trichomes L⁻¹ and 889 trichomes L⁻¹ (99 ± 176 trichomes L⁻¹, n = 34), showing relatively large spatial variability (Fig. 7A). Depth profile of *Trichodesmium* abundance along the H17-J82 transect is shown in Fig. 7B. *Trichodesmium* could not be detected through the water column at Sta. H14. In contrast, for Sta. H17, *Trichodesmium* could be detected through the water column and slightly increased toward the bottom. We observed relatively high *Trichodesmium* abundance in surface waters (86 trichomes L⁻¹ at ~ 1 m) for Sta. H12 along the H17-J82 transect.

Trichodesmium was mainly present as individual trichomes and colonial forms were rare. In the Beibu Gulf waters southwest off Hainan Island, *Trichodesmium* was present mainly (> 90% cell abundance) as free trichomes. By comparison, the present frequency of *Trichodesmium* colonies in east off Hainan Island increased to 42%, although the colonies were generally small (5-30 trichomes). *Trichodesmium hildebrandtii* were the dominant (cell abundance > 76%) species for *Trichodesmium* biomass during our sampling period.

N₂ fixation rate

Surface N_2 fixation rate ranged between 0.1 nmol N L^{-1} d^{-1} and 5.6 nmol N L^{-1} d^{-1} (1.0 ± 1.0 nmol N L^{-1} d^{-1} , n = 50) under nonbloom conditions (Fig. 8A). The minimum and maximum were observed at Sta. D19-5 and Sta. D13-10, respectively. We classified the stations into three groups based on bottom depth, i.e., I (<50 m), II (50-100 m), and III (>100 m). There is a general increasing trend of surface N₂. fixation rate offshore, i.e., from 0.6 ± 0.5 nmol N L⁻¹ d⁻¹ (station group I, n = 10), 0.8 ± 0.6 nmol N L⁻¹ d⁻¹ (station group II, n = 16) to 1.2 ± 1.2 nmol N L⁻¹ d⁻¹ (station group III, n = 24). While for the bloom Sta. D15-12, surface N₂ fixation rate (127.8 nmol N L⁻¹ d⁻¹) increased by 2-3 orders of magnitude higher than the nonbloom stations. Volumetric N₂ fixation generally decreased with depth in the upper water column (Fig. 9A). The highest values were usually encountered near surface, and two profiles (Sta. H17, J82) had elevated rates below the surface (Fig. 9A). Depthintegrated N₂ fixation rate (INF) ranged between 7.5 µmol N m² d⁻¹ and 163.1 $\mu mol~N~m^2~d^{-1}~(46.4\pm46.9~\mu mol~N~m^2$ d^{-1} , n = 11), and higher values (relative to the J57-J61 transect) were encountered along the H17-J82 transect south off Hainan Island (Fig. 9A).

Primary production (PP)

Surface PP ranged between 0.3 μ mol C L⁻¹ d⁻¹ and 23.6 μ mol C L⁻¹ d⁻¹ (2.9 ± 4.3 μ mol C L⁻¹ d⁻¹, *n* = 49) in the whole study area. Much higher PP was found in the coastal waters, with the highest rates encountered close to the Qiongzhou Strait (Fig. 8B). Being different from N₂ fixation distribution pattern, there is generally a decreasing trend off-shore, from 8.8 ± 7.7 μ mol C L⁻¹ d⁻¹ (station group I, *n* = 9), 2.3 ± 2.5 μ mol C L⁻¹ d⁻¹ (station group II, *n* = 16) to 1.1 ± 0.6 μ mol C L⁻¹ d⁻¹ (station group III, *n* = 24). Such spatial pattern of PP is consistent with MODIS remote sensing chlorophyll image during the sampling period, with much elevated chlorophyll concentrations near the



Fig. 7. *Trichodesmium* abundance in (A) surface water over the whole study area and (B) the water column along H17–J82 transect. Surface *Trichodesmium* abundances at each station are represented by the solid circles, with the circle area being proportional to the abundance. The square denotes the bloom Sta. D15-12 (2797 trichomes L^{-1} for *Trichodesmium*). The cross represents Sta. H14 where no *Trichodesmium* was detected.



Fig. 8. Distributions of (A) N₂ fixation and (B) PP rates in surface ($\sim 1 \text{ m}$) water. Rates of N₂ fixation (nmol N L⁻¹ d⁻¹) and PP (μ mol C L⁻¹ d⁻¹) at each station are represented by the solid circles, with the area being proportional to the rates measured. The blooming Sta. D15-12 was not included.

Qiongzhou Strait compared to the open waters (Fig. 5B). Consistently, previous studies on phytoplankton in the SCS have also revealed that the phytoplankton abundance in the waters east of the Qiongzhou Strait was several times higher than the SCS basin (Ning et al. 2004). Volumetric PP rates generally decreased with depth, and reached a minimum at the sampling bottom layer (Fig. 9B). This is consistent with the ordinary vertical pattern observed in the SCS (Chen and Chen 2006; Song et al. 2012). Depth-integrated PP rates (IPP) ranged between 8.9 mmol C m⁻² d⁻¹ and 88.0 mmol C m⁻² d⁻¹ at the vertically sampled stations (41.6 ± 29.5 mmol C m⁻² d⁻¹, n = 6, figure not shown). IPP values along the H17-J82 transect (19.1 ± 9.4 mmol C m⁻² d⁻¹, n = 3) were much

lower compared to the J57-J61 transect (64.0 \pm 24.1 mmol C m⁻² d⁻¹, n = 3). This pattern is opposite to that of INF distribution. The contrasting spatial patterns either for surface or depth-integrated rates indicate an uncoupling between N₂ fixation and PP.

Discussion

Variations in N_2 *fixation rate*

This is the first report of N_2 fixation rate using ${}^{15}N_2$ tracer assay in the NWSCS to the best of our knowledge. Values of N_2 fixation rate in this study generally fall in the published value range under nonbloom conditions in other areas of



Fig. 9. Depth profiles of (A) N₂ fixation rate and (B) PP.



Fig. 10. (A) Relative contribution of N₂ fixation to PP N demand in surface waters. (B) Box plot of contribution of N₂ fixation to PP N demand (NFR: N₂ fixation rate; PP: primary production) within three station groups (I, II, and III). In Fig. 10A, values of N₂ fixation/primary N demand at each station are represented by the solid circles, with the area being proportional to the ratios (legend shown in the inset box). In Fig. 10B, the stations are classified into three groups based on their bottom depths: I (<50 m), II (50-100 m), and II (>100 m). Outliers of 5% and 95% are shown in the box plot in Fig. 10B.

the SCS, including the SCS basin and the bordering Kuroshio upstream (Chen et al. 2008, 2014; Zhang et al. 2011), and the Vietnamese coastal upwelling further south (Voss et al. 2006; Grosse et al. 2010). Moreover, we propose that diazotrophic blooms may present an important contributor to N₂ fixation flux to the SCS. There are mainly two reasons. First, although diazotrophic blooms have never been reported in this area (of which the lack of sampling should be an important cause), Trichodesmium has been observed to bloom during summer in the adjacent Daya Bay (22.50°N, 114.60°E) which has similar oceanographic conditions to our sampling area (Li et al. 2008). Second, both results of this study and N₂ fixation rate during diazotrophic blooms can be increased by several orders of magnitude (about three orders of magnitude higher as observed in this study). Undoubtedly, future studies with high spatial and temporal resolutions are needed for better understanding the distribution pattern of N₂ fixation in the tropical/subtropical coastal areas, which are generally undersampled so far. The rare appearance of Trichodesmium colonies and the few trichomes per colony once present is consistent with previous observations in the northern SCS during summer (Wu et al. 2003; Chen et al. 2008). We calculate Trichodesmium trichome-specific N2 fixation rate of 22.8 pmol N trichome⁻¹ d⁻¹ at the bloom station D15-12. This value is in the upper range for Trichodesmium trichome-specific N2 fixation rate (2-26 pmol N trichome⁻¹ d⁻¹) measured in the SCS (Chen et al. 2008). It should be noted that the specific ability of N₂ fixation for Trichodesmium itself is subject to multiple environmental conditions and can be highly variable (Carpenter and Capone 1992; Capone et al. 2005), thus it may not be appropriate to extrapolate such rate across a large spatial extent. However, our results add to the background knowledge of N₂ fixation in the least-studied NWSCS.

Interestingly, our finding is somewhat different from that observed in the Benguela Upwelling region in the South Atlantic (Sohm et al. 2011*a*). We observed relatively low N₂ fixation rates in the coastal upwelling waters, but much higher rates in the transition zone between the coastal upwelling and the oligotrophic deep basin (Fig. 8). By comparison, highest N₂ fixation rates were seen in or near the Benguela Upwelling (Sohm et al. 2011*a*). Although the exact reason for the observed difference is not clear, it may at least indicate the variability of N₂ fixation response to physical forcings in different coastal upwelling regimes and highlight the necessity of more studies globally to better achieve an overall understanding.

N_2 fixation as a contributor to phytoplankton production

Diazotrophs may not be a major contributor to fuel phytoplankton production, as N2 fixation can meet 0.01-2.52% $(0.53 \pm 0.55\%, n = 49)$ N demand for PP after Redfield stoichiometry (C : N = 6.6) in the whole study area (Fig. 10A). Similarly, ratios of INF/IPP ranged between 0.12% (Sta. J59) and 6.52% (Sta. H12) and averaged 2.24%. The relative contribution of N₂ fixation to PP generally increased toward the basin (Fig. 10). Direct measurements of field NO₃⁻ uptake rates (NO₃⁻-based new production) in this area are still lacking. If we adopt the summertime f-ratio (= NO_3^- -based new production/PP) of ~ 0.20 in the northern SCS continental shelf (Chen and Chen 2006), it can be estimated that N_2 fixation may generally account for no more than 10% NO₃⁻based new production after Redfield stoichiometry. Obviously, upwelled NO_3^- from water column below is the dominant new N source sustaining production rather than N₂ fixation in the NWSCS under nonbloom conditions. This is consistent with the finding in the Vietnamese upwelling of southern SCS (Voss et al. 2006; Grosse et al. 2010). The ratios of N₂ fixation to phytoplankton production in this study generally fall in the range of values reported in other parts of the SCS, including the Vietnamese upwelling (10-13°N) further south (Voss et al. 2006), the SCS basin, and the bordering upstream Kuroshio (Chen et al. 2008). Though the ratios are not large, the role of N₂ fixation in marine biogeochemical cycling is in fact disproportionally important. There are mainly three reasons. First, N₂ fixation represents a contributor to net sequestration of atmospheric CO₂ by ocean compared to vertical NO₃⁻ supply (Karl et al. 2002). Second, the ratio of N₂ fixation to production is probably underestimated in this study, as recent studies have shown that the widely applied bubble injection in ¹⁵N₂ tracer assay will result in underestimation of N2 fixation rates mainly due to the slow dissolution process for N2 gas bubble and the subsequent isotopic equilibrium (Mohr et al. 2010). Last but not least, the occasional occurred but rarely captured diazotrophic blooms may represent an external N input that can not simply be ignored, further highlighting the possible underestimation of N₂ fixation contribution. Undoubtedly, future studies integrating advanced techniques and high spatio-temporal resolution are necessary for better evaluating the role of N₂ fixation in sustaining local production N demand.

Toward understanding N_2 fixation in the upwelling regimes: A physical-biological coupling perspective

Coastal upwelling may have played a major forcing of N₂ fixation via physical-biological coupling in the NWSCS. It has been revealed that coastal upwelling plays a decisive role in selecting phytoplankton species in the northern SCS coastal waters (Ning et al. 2004). Like other tropical/subtropical coastal upwellings, the NWSCS coastal upwelling (including waters around Hainan Island) has phytoplankton community dominated by coastal diatoms (cell abundance \sim 90%), and the dominant species generally include Chaetoceros spp., Thalassionema spp., Rhizosolenia spp., and Skeletonema spp., etc, during summer (Ke et al. 2011; Ling et al. 2012). As for our sampling area, coastal upwelling of subsurface water contains approximately 10 μ mol L⁻¹ NO₃⁻ (Figs. 3F, 4D). To date, there is only one published dFe depth profile in the SCS (at Sta. SEATS), showing that intermediate and deep waters have < 1 nmol L⁻¹ dFe (Wu et al. 2003). The observed near-full drawdown of macronutrients (NO₃ and PO_4^{3-}) along with relatively abundant dFe (several nmol L^{-1}) left in surface water (Fig. 6) probably indicates additional supply of Fe rather than solely from the source SCS deep water. We suggest that coastal upwelling should have played a major role in regulating dFe distribution in the NWSCS. Indeed, previous studies have confirmed that during coastal upwelling, upwelling source waters will be enriched in Fe, due to the contact with shelf sediments (Johnson et al. 1999). Moreover, due to the organic matter oxidation and resultant oxygen decrease in the continental shelf sediments, there may be a large dissolved Fe(II) flux out of the sediments and upwelled to surface ocean (Elrod et al. 2004). N_2 fixation rates show no regular correlation with dissolved iron concentrations, indicating that dissolved iron was not playing a leading role in regulating rates of N_2 fixation in these waters. Interestingly, a study in the tropical/subtropical Benguela Upwelling in South Atlantic did found that N_2 fixation rates were positively correlated to surface dFe concentrations, though the mechanism remains unclear either (Sohm et al. 2011*a*). It may indicate the difference in biogeochemistry that may largely influence N_2 fixation among coastal upwelling regimes. The presence of abundant organic ligands in this coastal upwelling regime (Ma et al. 2011) may have probably played an important role in regulating iron cycling, given that such ligands are much less abundant in the SCS basin area (Wu et al. 2003).

With relatively abundant dFe, phosphorus may become increasingly important in regulating N₂ fixation (Sanudo-Wilhelmy et al. 2001). Globally, magnitude and distribution of oceanic N₂ fixation have been suggested to be largely influenced by N : P nutrient utilization of phytoplankton (Karl et al. 2002; Mills and Arrigo 2010; Ward et al. 2013). Coastal upwellings should be an ideal environment to study such interactions. This may also hold true for the NWSCS which is under profound influence of coastal upwelling. As the major contributor of inorganic nutrients, the upwellingsource SCS deep water has a molar NO_3^- : PO_4^{3-} ratio of ~ 15.7 : 1 (Fig. 11, this study; X. Guo pers. comm.), which is close the Redfield N : P ratio. As stated above, N₂ fixation is not a major contributor to phytoplankton PP inorganic N demand. Thus, phytoplankton utilization, rather than N₂ fixation, should be the dominating biological process that regulates nutrient conditions in coastal upwelling surface waters. With the abundant upwelled nutrients, phytoplankton growth will be greatly stimulated in the coastal upwelling waters (Fig. 8B; Ke et al. 2011; Ling et al. 2012). Under this circumstance, the dominant fast-growing diatoms should have utilized macronutrients rapidly and caused the rapid drawdown of macronutrients in coastal waters. Both field and laboratory studies have confirmed that non-Redfield nutrient utilization is common for phytoplankton, with N : P utilization ratios being below Redfield during blooms, but above Redfield ratio in oligotrophic regions dominated by picoplankton (Geider and LaRoche 2002; Krauk et al. 2006; Mills and Arrigo 2010). It has been proposed that such plasticity is probably due to greater allocation to P-rich assembly machinery and exhibit lower cellular N : P ratios for fastgrowing cells, while resource-limited cells favor greater allocation to N-rich resource-acquisition machinery and, therefore, exhibit higher cellular N : P ratios (Geider and LaRoche 2002). Thus, P limitation for the growth of phytoplankton should have occurred before (or getting more severe than) N became limiting in the coastal upwelling and nearby waters. Indeed, multiple lines of evidence (nutrient enrichment bioassays, ³³P-based phosphate turnover times, etc.) have been



Fig. 11. Relationship between concentrations of nitrate and phosphate using all the data during the cruise. Note that more sampling data points relative to N₂ fixation measurements were obtained, but the stations are within the same sampling region. Most data points plotted are from 30 m and below, as phosphate concentration in the above water column are generally below detection limit (0.02 μ mol L⁻¹). Nutrient data are provided by the program of Chinese Offshore Investigation and Assessment.

demonstrated that P, rather than N, is the limiting nutrient for phytoplankton production in the northern SCS coastal waters (Xu et al. 2008). As a result, ecosystems dominated by rapidly growing phytoplankton with low N : P uptake ratios will greatly reduce the P available for any slowly growing diazotrophs (Mills and Arrigo 2010). Although we did not examine diazotrophic composition in this study, we propose that Trichodesmium are possibly the main N₂ fixers in coastal upwelling in the NWSCS. There are mainly two lines of clues. First, studies in the southern SCS have revealed that diazotrophic community is characterized by relatively low diversity within the Vietnamese upwelling, with Trichodesmium being by far the most abundant diazotrophs compared to either the unicellular or diatom-diazotrophic associations (Moisander et al. 2008; Grosse et al. 2010). Second, a sizefractionation experiment of N2 fixation rate conducted in another cruise (2013 summer) has also demonstrated that N₂ fixation was sustained substantially (~ 100%) by > 10 μ m diazotrophs in surface waters for coastal (<50 m) stations (Zhang et al. unpubl.). However, though probably dominating diazotrophic community in coastal waters, Trichodesmium spp. may only contribute a relatively small fraction of phytoplankton biomass. When assuming a mean carbon content of 3.5 pmol C cell⁻¹ for *Trichodesmium* spp. (Goebel et al. 2008), one can roughly estimate mean fraction of Trichodesmium spp. to total concentration of particulate organic carbon (POC, average = 7 μ mol L⁻¹ for the stations < 50 m; Zhang et al. unpubl.) to be less than 1%, implying that *Trichodesmium* was outcompeted by nondiazotrophic phytoplankton in dominating phytoplankton community under nonbloom conditions. To sum up, diazotrophs will unlikely take advantage in the competition with fast-growing diatoms for P acquisition in such coastal upwelling regimes (Mills and Arrigo 2010; Ward et al. 2013).

With the offshore Ekman transport of surface waters from coastal upwelling, surface waters will become progressively depleted in dissolved nutrients due to the imbalance of biological consumption and weakened replenishment (elevated stratification, reduced vertical supply, etc). Consequently, N gradually became the primary limiting nutrient in the SCS basin, contrasting with the coastal upwelling and inner shelf waters during summer (Chen et al. 2004; Xu et al. 2008). This is evidenced by the relatively long ³³P-based phosphate turnover times (generally > 4 d for either algal or bacterial fraction) in the SCS basin during summer, suggesting that without additional input other than recycling, the P supply may sustain more than 4 d of production (Xu et al. 2008). In other words, the longer turnover times of phosphate indicates that P was sufficient relative to the relatively low demand of phytoplankton growth in surface waters of the SCS basin during summer, making N nutrient limited for phytoplankton production. This idea is also supported by results of nutrient enrichment bioassays (Chen et al. 2004; Xu et al. 2008). Such N-limited but P-"relatively sufficient" nutrient status probably will facilitate N2 fixation relative to PP (Fig. 8). Similarly, the study in the Vietnamese upwelling region also found that volumetric N2 fixation rates were higher in N-limited compared to N-replete conditions (Grosse et al. 2010). The possibility for diazotrophs to utilize dissolved organic phosphorus as an alternative P source under phosphorous scarcity (Dyhrman et al. 2006) should also be considered in future studies.

Schematic physical-biological coupling on N₂ fixation in coastal upwelling in the NWSCS is summarized in Fig. 12. As shown, coastal upwelling waters are characterized by high PP and low N₂ fixation rate, as N₂ fixation is generally hindered in the coastal water where upwelling brings in replete nutrients (Fe, N, P) and preferentially facilitate nondiazotrophic fast-growing diatoms (low N : P utilization ratios), resulting in P deficiency. With its offshore Ekman transport, the transition waters between coastal upwelling and oligotrophic deep basin will become progressively depleted in nutrients due to weakened replenishment, and N gradually became the primary limiting nutrient (while dFe is relatively replete at level > 1 nmol L^{-1} and may not be limiting) for PP. This transition environment is more favorable for N₂ fixation relative to coastal upwelling and characterized by lower PP and elevated N2 fixation. Finally, in the remote deep basin, both dFe and N may become depleted and limit either N₂ fixation or PP. Besides of nutrient regulation, some other key environmental conditions in the coastal upwelling, such as decreased water column stability and lower



Fig. 12. Schematic of physical-biological coupling in coastal upwelling and its adjacent waters. Abbreviations are as follows: $NFR = N_2$ fixation rate; PP = primary production.

water temperature, may also partly contribute to the variability of N₂ fixation. For example, more stable water column and warmer seawater turns to be more favorable for N2 fixation in offshore waters. In the northern SCS deep basin, there seems a correlation of high Trichodesmium abundance with internal waves, but the exact reason for such a possible link remains to be clarified (Shiozaki et al. 2014). A correlation between N2 fixation rates and water column vertical stability (density gradient) have been reported in the subtropical ECS continental shelf (Zhang et al. 2012). In the North Pacific subtropical gyre, N₂ fixation and diazotroph community structure have been found to be tightly linked to eddy-eddy interaction, suggesting the significant role of physical forcing on N₂ fixation (Church et al. 2009). As suggested by Subramaniam et al. (2013), the magnitude of N_2 fixation is probably dependent on the local upwelling regime and time varying. Considering the fact that coastal upwellings have great spatio-temporal variability subject to climate (Bakun 1990), it is with no doubt that more studies are in emergent need to better unveil the effect of such physical forcing of N₂ fixation with global implications.

N₂ fixation as an external N source

As stated above, N₂ fixation represents an external N source and contributes to net sequestration of atmospheric CO₂ by the ocean (Karl et al. 2002). In contrast, the upwelled or diffusive supply of NO₃⁻ is generally accompanied by an associated amount of CO₂, resulting in a much reduced net removal of atmospheric CO₂. If we extrapolate the areal N₂ fixation rate (mean 46.4 μ mol N m⁻² d⁻¹) to the whole northwestern SCS shelf (area = 33 × 10⁴ km², north of 17°N), we estimate that summertime (90 d) N₂ fixation may

contribute external N at a flux of 1.4 Gmol N under nonbloom conditions. Besides of N₂ fixation, there are mainly two other external N sources, i.e., the atmospheric deposition and riverine input of N, that also contribute to net sequestration of atmospheric CO₂. Direct results of atmospheric inorganic N deposition for the SCS are not abundant. Based on literature results, daily atmospheric inorganic N flux of $\sim 105 \ \mu mol \ N \ m^{-2} \ d^{-1}$ in the SCS can be derived when the seasonal variability was not taken into account (Kim et al. 2014; references therein). Thus, the extrapolated atmospheric inorganic N deposition flux would be 3.2 Gmol N for the northern SCS shelf in summer. The dominant land river that influences the northern SCS is the Zhujiang, the second largest river in China (after Changjiang) and ranks 13th in the world in respect of freshwater discharge. The Pearl River discharges DIN to the northern SCS at an annual flux of 26 Gmol N, and \sim 75% total annual water flow occurs during the wet season April-September (Liu et al. 2009). Based on these values, a summertime riverine dissolved inorganic nitrogen (DIN) flux of 9.8 Gmol N can be roughly estimated. Finally, we estimate that N₂ fixation may account for about 9.7% of external N sources to the northern SCS shelf, following the contribution of riverine input (68.1%) and atmospheric deposition (22.2%). However, the relative contribution of N2 fixation in most offshore waters in the northern SCS should be in fact much more important than that ratio itself, mainly due to two reasons. First, dramatic impact of Pearl River nutrient input on biogeochemistry was basically confined to the freshwater plume in the inner shelf east of \sim 114°E, north of \sim 21°N during southwesterly monsoon season (Cai et al. 2004). It has implications because this could also hold true for another major

land river of the SCS, i.e., Mekong River. Thus, N_2 fixation and atmospheric deposition, though in smaller fluxes, may in fact have together played a much more important role in fueling production relative to riverine input over most of the SCS open waters. Nevertheless, it should be of significant implications to conduct samplings north of this study to examine the possibly profound effect of Pearl River plume on N_2 fixation, as observed in some other major tropical/ subtropical land rivers (Voss et al. 2006; Subramaniam et al. 2008). Second, N_2 fixation rate may be underestimated due to methodological reason as suggested by recent studies (Mohr et al. 2010). Obviously, more studies should be undertaken in the undersampled SCS, to better understand the controlling factors and the biogeochemical significance of N_2 fixation.

It is noteworthy that the relative contributions of external N sources may not necessarily be representative of the subtropical upwelling regions worldwide, as each contributor should have large variability among different environments. Comprehensive studies are necessary to approach that goal for any specific region. However, a perspective of physicalbiological coupling may be of common implications for tropical/subtropical coastal upwelling and the adjacent waters.

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